Nickel Reference Exposure Levels

NICKEL AND NICKEL COMPOUNDS. NICKEL OXIDE. REFERENCE EXPOSURE LEVELS (RELs)

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

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Contents

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1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8 hour and chronic reference exposure levels (RELs) and was adopted in December 2008. The TSD presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop acute, 8-hour and chronic RELs for nickel and nickel compounds. This document will be added to Appendix D of the TSD.

Nickel (II) causes a variety of non-carcinogenic toxic effects including occupational contact dermatitis, occupational asthma, and reproductive toxicity in humans. Studies in experimental animals exhibit immune suppression, nephrotoxicity, pneumotoxicity, perinatal mortality and altered gene expression. The most sensitive effects appear to be in the lung and immune system. The key values are summarized below.

Molecular Formula	Molecular Weight	Synonyms	CAS Registry Number
Ni	58.69	elemental nickel nickel metal	7440-02-0
NiO	74.69	nickel oxide green nickel monoxide nickel(II) oxide	1313-99-1
Ni_2O_3	165.36	nickel oxide black	
$Ni(OH)_2$	92.71	nickel hydroxide nickelous hydroxide	12054-48-7
$NiCl_2$	129.6	nickel chloride nickel dichloride	7718-54-9
$NiSO_4$	154.75	nickel sulfate nickelous sulfate	7786-81-4
$NiSO_4 \cdot 6H_2O$	262.85	nickel sulfate hexahydrate	10101-97-0
$NiCO_3$	118.7	nickel carbonate carbonic acid nickel(2+) salt nickelous carbonate	3333-67-3

Molecular Formula	Molecular Weight	Synonyms	CAS Registry Number
Ni_3S_2	240.19	nickel subsulfide trinickel disulfide Heazlewoodite	12035-72-2
NiS	90.77	nickel sulfide nickel monosulfide Millerite	11113-75-0
$Ni(NO_3)_2 \cdot 6H_2O$	290.81	nickel nitrate hexahydrate	13478-00-7
$Ni(O_2CCH_3)_2$	178.78	nickel acetate	373-02-4
$Ni_3(CO_3)(OH)_2$	304.12	nickel carbonate hydroxide	12607-70-4

1.1 Acute Toxicity (for a 1-hour exposure)

Inhalation reference exposure level	**1.1 μg Ni/m^3**
Critical effect(s)	Small decrements in airway function tests, especially in asthmatics
Hazard Index target(s)	Respiratory System; Immune System

1.2 8-Hour REL (for repeated 8-hour exposures)

Inhalation reference exposure level	**0.08 μg Ni/m^3**
Critical effect(s)	Immunotoxicity, lung lesions
Hazard Index target(s)	Respiratory System; Immune System

1.3 Chronic REL Nickel and Nickel Compounds (except NiO)

Inhalation reference exposure level	**0.015 μg Ni/m^3**
Critical effect(s)	Lung, nasal epithelial and lymphatic pathology in male and female rats
Hazard index target(s)	Respiratory system; hematopoietic system

1.4 Chronic REL Nickel Oxide

Inhalation reference exposure level	**0.06 μg Ni/m^3**
Critical effect(s)	Lung pathology in male and female mice
Hazard index target(s)	Respiratory system

2. Physical and Chemical Properties (HSDB, 1994 except as noted)

Description	Ni metal: silvery metal NiO: black crystals $NiCl_2$: yellow deliquescent crystals (U.S.EPA, 1985)
Density	8.9 g/cm^3 (Ni) 2.07 g/cm^3 ($NiSO_4 \cdot 6H_2O$) 6.67 g/cm^3 (NiO)
Boiling point	2730°C (Ni)
Melting point	1455°C (Ni); 1030°C ($NiCl_2$)
Vapor pressure	not applicable for dust
Flashpoint	not applicable
Explosive limits	Nickel dust or powder is flammable (CDTSC, 1985).
Solubility	Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water-soluble.
Odor threshold	odorless
Metabolites	Ni^{2+}
Oxidation states	0, +1, +2, +3 (Von Burg, 1997)
Conversion factor	1 ppm = 2.4 mg/m^3 (Ni metal) (Von Burg, 1997)

3. Major Uses or Sources of Exposure

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent (Ni(II) or Ni^{2+}) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, about 40% of nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). About 20% of nickel is produced as nickel sulfate and hydroxide used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985, Von Burg, 1997). Nickel dust or powder is flammable (CDTSC, 1985). Nickel carbonyl also is airborne. However, because of its unique toxicity relative to the inorganic nickel compounds, this REL is not applicable to nickel carbonyl.

3.1 Air

The primary stationary source categories that emit nickel into ambient air in California are fuel combustion, nickel alloy manufacture, cement production, asbestos mining and milling, municipal waste sludge incineration, iron and steel foundries, secondary metal recovery, cooling towers, coal gasification petroleum processing, and electroplating. Also nickel has been detected in vehicular exhaust, tobacco smoke, and indoor smoke from home-heating and cooking fuels (CARB, 1991). The United States Environmental Protection Agency (U.S. EPA, 1986) estimated that particles found in ambient air as a result of oil combustion might contain nickel in the form of nickel sulfate, with smaller amounts of nickel oxide and complex metal oxides containing nickel. The majority of the nickel in the atmosphere is thought to be associated with human activities. Up to one-third of atmospheric nickel could come from windblown dusts, forest fires and volcanic emissions (CARB, 1991). The annual statewide average ambient air concentration of nickel for 2002 was 4.5 ± 4.1 SD ng/m^3 (CARB, 2008). This value is quite similar to values reported for earlier years 1992 to 2001 (CARB, 2008). Besides ambient and occupational exposures, nickel is present in mainstream cigarette smoke in concentrations higher than other metals such as copper, cadmium and iron: 0.2-0.51, 0.19, 0.07-0.35, and 0.042 μg/m^3, respectively (IARC, 1986).

3.2 Soil

Nickel occurs naturally in the Earth's crust at an average concentration of 0.0086% (86 ppm) (Duke, 1980). The nickel content of soil can vary widely depending on local geology. Both the southeastern United States and southern Quebec can have nickel concentrations greater than 1000 ppm due to local ultramafic rock, which is rich in nickel. Typical nickel soil concentrations range from 4 to 80 ppm (ATSDR, 2005). A soil survey by the U.S. Geological Survey throughout the U.S. reported concentrations from <5 to 700 ppm, with a geometric mean of 13.0 ± 2.31. Nickel ranked 15th among 50 elements included in the study (Shacklette and Boerngen, 1984). Auto emissions can also raise the level of nickel in soil. Lagerwerff and Sprecht (1970) found nickel concentrations from 0.9 to 7.4 ppm in roadside soils. The concentrations were lower at greater distances from the road and at greater soil depths.

3.3 Water

Nickel enters groundwater and surface water via dissolution of rocks and soils, from atmospheric deposition, from biological decay, and from waste disposal. Nickel compounds are relatively soluble in water, especially at pH values less than 6.5, and usually exist as nickel ions in aqueous environments. Uncontaminated surface freshwater and seawater usually contain low concentrations of nickel (<0.3 μg/L, Barceloux, 1999). The nickel concentration of fresh surface water has been reported to average between 15 and 20 μg/L (Grandjean, 1984; ATSDR, 2005). The nickel concentration in groundwater is normally less than 20 μg/L (U.S.EPA, 1986), and levels appear similar in raw, treated, and distributed municipal water.

Elevated nickel in drinking water may result from corrosion of nickel-containing alloys used in valves and other components in the water distribution system as well as from nickel-plated faucets. Tap water that is used for drinking purposes generally contains nickel at concentrations ranging from 0.55 to 25 μg Ni/L in the United States (ATSDR, 2005; FDA 2000; O'Rourke et al. 1999; Thomas et al. 1999). Nickel concentrations in tap water measured in the Total Diet Study 1991–1999 ranged from 0 to 0.025 mg Ni/kg (0–25 μg Ni/L) with a mean value of 0.002 mg/kg (2 μg Ni/L) (FDA 2000). Analysis of data obtained during 1995 - 1997 from the National Human Exposure Assessment Study (NHEXAS) yielded median concentrations of nickel in tap water (used as drinking water) of 4.3 μg Ni/L (10.6 μg Ni/L, 90th percentile) in the Arizona study and 4.0 μg Ni/L (11 μg Ni/L, 90th percentile) in the U.S. EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin) study (O'Rourke et al., 1999; Thomas et al., 1999). In a 1969–1970 survey of 969 water supplies in the United States representing all water supplies in eight metropolitan areas and one state (2,503 samples), 21.7% of samples had concentrations <1 μg Ni/L, 43.2% of the samples contained between 1 and 5 μg Ni/L, 25.6% of the samples contained between 6 and 10 μg Ni/L, 8.5% of the samples contained between 11 and 20 μg Ni/L, and 1% had levels >20 μg Ni/L (NAS 1975).

Nickel has been detected in California drinking water sources. According to the monitoring data collected by the California Department of Health Services (DHS) between 1984 and 1997, the highest, average and median concentrations of nickel in water were 540 μg/L, 26 μg/L, and 17.9 μg/L, respectively (DHS, 1998). The detection limit for the purposes of reporting for nickel is 10 μg/L (10 ppb).

3.4 Food

Terrestrial plants take up nickel from soil mainly via the roots. The amount of uptake depends on the concentration in soil, soil pH, organic matter content and the type of plant. The nickel concentration in most natural vegetation ranged from 0.05 to 5.0 mg Ni/kg dry weight (dw) (NRC, 1975). Some food sources such as chocolate, nuts, beans, peas, and grains are relatively rich in nickel.

There have been several studies regarding nickel content in an average diet (ATSDR, 2005). Current information on the dietary intake of nickel in the United States is based on data gathered from the NHEXAS study. Nickel concentrations were measured in duplicate diet samples, which, in combination with study participant's estimates of food and water intake, were used to determine both the overall concentration of nickel in combined solids and liquids in the total diet and the average nickel intake of study participants. In the U.S. EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin) study, the mean and median concentrations of nickel in combined dietary solids and liquids were 47 and 43 μg Ni/kg, respectively (Thomas et al., 1999).

Calamarie et al. (1982) showed that nickel is not likely to accumulate in fish. They exposed rainbow trout (*Salmo gairdneri*) to nickel contaminated water at 1.0 mg Ni/L for 180 days and found 2.9 mg Ni/kg wet weight in liver, 4.0 mg/kg in kidneys, and 0.8

mg/kg in muscle. Initial study values for these tissues were 1.5, 1.5, and 0.5 mg Ni/kg, respectively.

Myron et al. (1978) studied nickel levels in meals sampled from the University of North Dakota and from a hospital. The average nickel concentration of the student meals ranged from 0.19 to 0.29 μg Ni/g dw and for the hospital meals from 0.21 to 0.41 μg Ni/g dw. Based on the nine diets examined, the authors estimated an average daily dietary intake of 168 ± 11 μg nickel. This value is similar to those estimated in other studies (ATSDR, 2005).

Rietschel et al. (2008) studied trends in nickel sensitivity in 25,626 North American subjects over the period 1992 to 2004. The data exhibited a steady increase in nickel sensitivity indicated by patch test from 14.5% in 1992 to 18.8% in 2004 ($P < 0.0001$). Females were 1.1 to 1.2 times more likely to be allergic in the late (2001-2004) group compared to the early group (1992-1995) with a relative risk (RR) = 1.2, 95% C.I. 1.10-1.28, $P < 0.0001$, or the middle group (1996-2000) $P = 0.0011$. Younger males and females (≤ 18 yr) showed significantly higher sensitivity compared to older subjects, i.e. 14.1% (55/389) vs. 6.1% (536/8839) in males and 32.4% (177/546) vs. 21.4% (3385/15,821) in females. The cause of increased sensitivity is unclear but seems indicative of increased population exposures to nickel possibly related to body piercing.

4. Toxicokinetics

4.1 Absorption

4.1.1 Oral route

Ishimatsu et al. (1995) demonstrated that the absorption fraction of orally administered nickel compounds in rats was closely related to their water solubility. They administered eight nickel compounds and nickel metal. The solubilities in saline solution were in the following order: [$Ni(NO_3)_2$ > $NiCl_2$ > $NiSO_4$] >> [NiS > Ni_3S_2] > [NiO (black) > Ni (metal) > NiO (green)]. The insoluble nickel metal and nickel oxides ranged from 0.01 to 0.09% absorbed. The absorption of the slightly soluble nickel subsulfide and nickel sulfide was 0.5% to 2.1% and the soluble nickel compounds (sulfate, nitrate and chloride) ranged from 10 to 34 percent. In rats administered $NiCl_2$, $NiSO_4$, and NiS 84-87% of recovered nickel was detected in the kidneys. Lesser kidney ratios were found for Ni_3S_2, $Ni(NO_3)_2$, NiO(B) and Ni(M): 76%, 73%, 62%, and 51%, respectively. However, NiO(G) showed greater recovery from liver than kidney.

Ho and Furst (1973) reported that intubation of rats with $^{63}NiCl_2$ in 0.1N HCl led to 3 to 6 percent absorption of the labeled nickel, independent of dose level (4, 16, and 64 mg Ni/kg body weight (bw)). One day after administration 94 to 97 percent of the dose was excreted in the feces and 3 to 6 percent in the urine. Nielsen et al. (1993) administered $^{57}NiCl_2$ at 3 to 300 μg Ni/kg bw by gastric intubation to male mice, and estimated that intestinal absorption ranged from 1.7 to 7.5 percent of administered dose.

Nickel is absorbed in the gastrointestinal (G.I.) tract of humans either as free ions or as complexes. The degree of uptake or bioavailability depends on the vehicle (water or food) and has ranged from 1% to 40% in several studies (Table 1). Cronin et al. (1980) reported that ingestion of a soluble nickel compound during fasting by a group of female subjects resulted in urinary elimination of four to 20 percent of the dose. Sunderman et al. (1989) found that about 40 times more nickel was absorbed from the GI tract when nickel sulfate was given to human volunteers in drinking water (27 ± 17 %, mean ± SD) than when it was given in food (0.7 ± 0.4 %). Sunderman et al. (1989) also reported that absorption fraction was independent of dose at 12, 18, or 50 μg Ni/kg bw.

Solomons et al. (1982) and Nielson et al. (1999) reported similar results. They found that plasma nickel concentrations in five fasted human subjects were significantly elevated when they were given nickel sulfate (5 mg Ni) in drinking water with a peak level of about 80 μg Ni/L at three hours after oral administration. When five mg Ni (as nickel sulfate) were administered in whole cow-milk, coffee, tea, orange juice, or Coca Cola®, the rise in plasma Ni was significantly suppressed with all but the Coca Cola®. By four days after administration, 26% of a dose given in water was excreted in urine and 76% in feces. When the nickel dose was given in food, 2% was excreted in the urine and the balance in feces. The elimination half-life for absorbed nickel averaged 28 ± 9 hours (Sunderman et al., 1989).

Solomons et al. (1982) showed that plasma nickel levels of subjects who consumed a typical Guatemalan meal with 5 mg nickel or a North American breakfast with 5 mg nickel were only about 5 to 20 percent of that which resulted from the consumption of 5 mg nickel in water. Nielsen et al. (1999) administered nickel in drinking water (12 μg Ni/kg bw) to eight fasted volunteers at different time intervals, with standardized portions of scrambled eggs. They found that the highest fraction of nickel dose (25.8%) excreted in urine was observed when the scrambled eggs were taken four hours prior to nickel in drinking water. A much lower fraction of nickel dose (2.5%) was observed when the nickel was mixed into the eggs or when the drinking water was taken together with the eggs (3.4%).

Table 1. Absorption of Ingested Nickel in Humans from Bioavailability Studies (Diamond et al., 1998; ATSDR, 2005)

Study	Number of subjects	Vehicle	Duration	Fasting status	Absorption (% of Dose)
Nielsen et al., 1999	8	Water plus scrambled eggs	Acute	Fasted	25.8 to 2.5
Patriarca et al., 1997	4	Water	Acute	Fasted	29-40
Sunderman et al., 1989	8	Water	Acute	Fasted	29.3
Sunderman et al., 1989	8	Food	Acute	Fasted	1.8
Cronin et al. , 1980	5	Capsule plus 100 mL water	Acute	Fasted	12-32
Christensen & Lagassoni, 1981	8	Capsule	Acute	With meal	5.7
Gawkrodger et al., 1986	3	Capsule	Acute	With meal	2.7, 2.8
Menne et al., 1978	6	Capsule	Acute	Not fasted	2.2 (women)
Menne et al., 1978	7	Capsule	Acute	Not fasted	1.7 (men)
Horak & Sunderman, 1973	10-50	Food	Chronic	Not fasted	1.0
McNeeley et al., 1972	19	Food & water	Chronic	Not fasted	1.6
McNeeley et al., 1972	20	Food	Chronic	Not fasted	1.2

Patriarca et al. (1997) studied nickel metabolism in humans using the stable isotope ^{62}Ni (98.83%, as metal). Four healthy adult subjects (two women and two men) were fasted overnight and administered 10 µg ^{62}Ni/kg bw in water. Blood samples were drawn in fixed intervals and the total daily output of urine and feces was collected for the first five days after dose ingestion. ^{62}Ni was measured in plasma, urine and feces by isotope dilution using ^{61}Ni and plasma-mass spectrometry. Fecal excretion of ^{62}Ni averaged 66.9 ± 4.9 % of administered dose with an absorbed fraction of 33.1 ± 4.9 %. Urinary excretion over five days ranged from 51% to 82% (mean ± SD= 65.2 ± 13.4 %) of absorbed dose. Plasma ^{62}Ni peaked between 1.5 and 2.5 hours after ingestion with concentrations ranging between 269 and 344 nM; ^{62}Ni was rapidly cleared from the plasma but was still detectable at 96 hr post ingestion (< 32 nM). The authors reported no evidence of biliary excretion or enterohepatic circulation of ^{62}Ni as indicated by the

appearance of secondary peaks in plasma or urinary nickel concentrations. Also the elimination of ^{62}Ni in feces followed the same pattern as the fecal marker (radio-opaque pellets) indicating that biliary excretion is very low or absent in humans, albeit with a limited number of subjects.

Nickel has been reported as an essential element in several animal species. Signs of Ni deficiency include depressed growth and reduced hematocrit (Nielsen, 1996). In the case of human nutrition the essentiality of Ni has yet to be established (IOM, 2001).

4.1.2 Inhalation route

Animal models have been used to estimate the inhalation absorption of water-soluble and water-insoluble nickel compounds. English et al. (1981) administered nickel chloride and nickel oxide intratracheally to rats and reported greater than 50% of the soluble nickel chloride was cleared from the lungs within three days. Most of the nickel was excreted in the urine. In contrast, the water-insoluble nickel oxide persisted in the lung for more than 90 days, and the nickel was excreted equally in urine and feces. Valentine and Fisher (1984) administered slightly soluble nickel subsulfide intratracheally to mice and observed the pulmonary clearance to have two distinct components with initial and final half-lives of 1.2 and 12.4 days, respectively. The excretion of the chemical (measured as ^{63}Ni) was 60% in the urine and 40% in the feces. Similar findings were reported by Finch et al. (1987) who observed that the pulmonary clearance of intratracheally administered nickel subsulfide in mice was biphasic with clearance half-lives of two hours and 119 hours for initial and final phases, respectively.

Tanaka et al. (1985) exposed male Wistar rats to NiO aerosols of mass median aerodynamic diameter (MMAD), 1.2 and 4.0 μm. The average exposure concentration was 0.6 mg/m^3 or 70 mg/m^3 and total exposure time was 140 hours. Some rats were sacrificed after exposure while others were kept for 12 and 20 months prior to sacrifice. The biological half-lives of NiO deposited in the lungs based on the assumption of first order clearance kinetics were 11.5 and 21 months for 1.2 and 4.0 μm MMAD aerosols, respectively. The relation used was $T_{50} = -0.301/\log(1- f)$, where f, the clearance ratio, was selected as 0.002 or 0.001 depending on fit to the experimental data.

Following a single 70-minute inhalation exposure of rats to green nickel oxide (^{63}NiO; 9.9 mg Ni/m^3; AMAD 1.3 μm), the fraction of the inhaled material deposited in the total respiratory tract was 0.13, with 0.08 deposited in the upper respiratory tract and 0.05 deposited in the lower respiratory tract (Benson et al. 1994). During the 180 days post-exposure, nickel was not detected in extra-respiratory tract tissues.

Tanaka et al. (1988) studied the biological half-life of amorphous NiS aerosols in exposed rats. The rats were exposed to a NiS aerosol with MMAD of 4.0 μm and either a single four hr exposure of 107 mg/m^3 or repeated 8.8 mg/m^3 for 7 hr/day, 5 days/week for one month. After exposure, the nickel contents in lung, liver, kidney, spleen, blood and urine were measured. In sharp contrast to the findings with NiO (above), NiS was rapidly cleared from lung tissue following a four-hour exposure with a half-life of 20

hours (f = 0.57). Repeated exposures of NiS at lower concentration showed no accumulation of NiS in the lung and similar clearance kinetics following the final exposure (their Fig. 2).

Following a single 120 minute inhalation exposure of rats to nickel subsulfide ($^{63}Ni_3S_2$; 5.7 mg Ni/m^3 AMAD 1.3 μm), the fraction of inhaled material deposited in the upper respiratory tract was similar to that observed for nickel oxide (0.14 in the total respiratory tract, 0.09 in the upper respiratory tract, and 0.05 in the lower respiratory tract). In contrast to nickel from nickel oxide, nickel from nickel subsulfide was detected in the blood, kidneys, and carcass between 4 and 24 hours after the exposure.

Data in rats and mice indicate that a higher percentage of less-soluble nickel compounds was retained in the lungs for a longer time than soluble nickel compounds (Benson et al. 1987, 1988; Dunnick et al. 1989; Tanaka et al. 1985) and that the lung burden of nickel decreased with increasing particle size (≤ 4 μm) (Kodama et al. 1985a, 1985b). Nickel retention was six times (mice) to 10 times (rats) greater in animals exposed to less-soluble nickel subsulfide compared to soluble nickel sulfate (Benson et al. 1987, 1988).

The lung burdens of nickel generally increased with increasing exposure duration and increasing levels of the various nickel compounds (Dunnick et al. 1988, 1989). From weeks 9 to 13 of exposure, lung levels of nickel sulfate and nickel subsulfide remained constant while levels of nickel oxide continued to increase (Dunnick et al. 1989). Slow clearance of nickel oxide from the lungs was also observed in hamsters (Wehner and Craig 1972). Approximately 20% of the inhaled concentration of nickel oxide was retained in the lungs at the end of exposure for two days, three weeks, or three months. The retention was not dependent on the duration of exposure or exposure concentration. By 45 days after the last exposure to nickel oxide (two-day exposure), 45% of the initial lung burden was still present in the lungs (Wehner and Craig 1972).

Workers occupationally exposed to nickel have higher lung burdens of nickel than the general population. Dry weight nickel content of the lungs at autopsy was 330 ± 380 μg/g in roasting and smelting workers exposed to less-soluble compounds, 34 ± 48 μg/g in electrolysis workers exposed to soluble nickel compounds, and 0.76 ± 0.39 μg/g in unexposed controls (Andersen and Svenes 1989). In an update of this study, Svenes and Andersen (1998) examined 10 tissue samples taken from different regions of the lungs of 15 deceased nickel refinery workers; the mean nickel concentration was 50 μg/g dry weight. Nickel levels in the lungs of cancer victims did not differ from those of other nickel workers (Kollmeier et al. 1987; Raithel et al. 1989).

Nickel levels in the nasal mucosa are higher in workers exposed to less-soluble nickel compounds relative to soluble nickel compounds (Torjussen and Andersen 1979). These results indicate that, following inhalation exposure, less-soluble nickel compounds remain deposited in the nasal mucosa. Higher serum nickel levels have been found in occupationally exposed individuals compared to non-exposed controls (Angerer and Lehnert 1990; Elias et al. 1989; Torjussen and Andersen 1979). Serum nickel levels were found to be higher in workers exposed to soluble nickel compounds compared to workers exposed to less-soluble nickel compounds (Torjussen and Andersen 1979).

Concentrations of nickel in the plasma, urine, and hair were similar in nickel-sensitive individuals compared to non-sensitive individuals (Spruit and Bongaarts 1977).

Serita et al. (1999) evaluated pulmonary clearance and lesions in rats after a single inhalation of ultrafine metallic nickel (Uf-Ni, 20 nm average particle diameter). Wistar rats (sex unspecified) were exposed to 0.15 (Low), 1.14 (Medium), or 2.54 (High) mg Uf-Ni/m^3 for five hours. Groups of five rats per dose group were sacrificed at 0 hr and 1, 3, 7, 14, and 21 days post exposure. The amount of nickel in the lung accumulated in a dose-dependent manner (1.4, 10.1, 33.5 µg Ni/lung, respectively). The half times for nickel in the lung averaged about 32 days and appeared independent of initial dose.

4.2 Distribution

Several studies of nickel administered to rodents via the oral route show that nickel was mainly concentrated in the kidneys, liver, and lungs, and the absorbed nickel was excreted primarily in the urine (Borg and Tjalve, 1988; Jasim and Tjalve, 1984, 1986a, 1986b; Dieter et al., 1988). Nielsen et al. (1993) showed that retention and distribution of nickel in mice was dependent on the route of administration. As shown in Table 2, Nielsen et al. (1993) showed that 20 hours after nickel administration, deposition in body tissues resulting from intraperitoneal (i.p.) injection was much greater than that observed after gavage administration.

Table 2. Median Nickel Body Burden and Contents of major Organs in Mice as Percentage of Administered Dose (from Nielsen et al., 1993)*.

Tissue	Gastric intubation	Intraperitoneal Injection
Liver	0.0439 (0.046)[a]	0.255 (0.044)[b]
Kidneys	0.029 (0.030)	1.772 (0.306)
Lungs	<0.010 (0.010)	0.114 (0.020)
Carcass	0.106 (0.111)	3.164 (0.546)
Stomach	0.014 (0.015)	<0.010 (0.002)
Intestine	0.762 (0.799)	0.490 (0.084)
Total body burden	0.954 (1.0)	5.794 (1.0)

*Note: a) Measurements made 20 hr after oral dose of 10 µmol Ni/kg bw. b) Measurements made 20 hr after intraperitoneal injection of 1.0 µmol Ni/kg bw. Values in parentheses are ratios of relative tissue burden over total body burden.

Ishimatsu et al. (1995) evaluated the distribution of various nickel compounds in rat organs 24 hours after oral administration. Male Wistar rats (10 weeks old, 8/compound) were administered the nickel compounds by gavage as 10 mg of Ni dissolved in a 5% starch saline solution. The animals were sacrificed at 24 hr after dosing and organs and blood taken for Ni determination. Selected results are presented in Table 3A. The kidney stands out as the major site of nickel deposition.

Table 3A. Mean Nickel Concentrations in Rat Organs 24 Hours after Oral Administration (adapted from Ishimatsu et al., 1995)*

Ni Compound	Lung µg/g	Liver µg/g	Kidney µg/g	Heart µg/g	Brain µg/g	Blood µg/mL
NiO (Green)	0.04	0.02	0.03	0.03	0.03	0.03
Ni metal	0.18	0.04	0.31	0.04	0.02	0.02
NiO (Black)	0.08	0.04	0.32	0.04	0.02	0.05
Ni_3S_2	0.17	0.07	1.2	0.04	0.02	0.05
NiS	0.34	0.11	6.4	0.60	0.04	0.21
$NiSO_4$	2.5	0.57	25.5	0.47	0.04	0.28
$NICl_2$	3.7	0.53	28.7	1.2	0.18	0.31
$Ni(NO_3)_2$	6.3	1.1	32.6	2.4	0.15	2.25
Control	0.04	0.03	0.03	0.03	0.02	0.03

* Note 8 animals/compound; 10 mg Ni oral dose by gavage

Obone et al. (1999) measured the accumulation of nickel in tissues of rats exposed to $NiSO_4$ in drinking water for 13 weeks. Accumulation in all organs examined was observed to increase with increasing dose level. The order of accumulation compared to the control was kidneys > testes > brain > spleen > lung = heart= liver (Table 3B).

Table 3B. Mean Nickel Concentrations in Rat Organs after 13 Weeks Exposure to $NiSO_4$ in Drinking Water (µg Ni/g tissue, Obone et al., 1999)*

Treatment $NiSO_4$	Liver	Kidney	Spleen	Heart	Lungs	Brain	Testis
0%	1.5806	1.3929	1.5105	1.6048	1.2153	1.5874	1.4993
0.02%	1.6024	1.8824	1.8476	1.7368	1.5961	1.6802	1.8519
0.05%	1.6310	3.4540	1.8592	1.8333	1.9512	1.7710	2.0531
0.1%	2.0842	5.4817	2.2610	2.1231	2.1120	2.7778	2.8369

*Note: Values are means of three different experiments. Measurements made 24 hr after termination of exposure.

Absorbed nickel is unlikely to exist as free ionic Ni^{2+}, but rather as nickel complexes. Sunderman and Oskarsson (1991) noted that in humans absorbed nickel is transported by binding to a metalloprotein (nickeloplasmin), albumin, and ultra-filterable ligands, such as small polypeptides and L-histidine. Van Soestbergen and Sunderman (1972) administered nickel chloride (as ^{63}Ni) to rabbits by intravenous injection at 0.24 mg Ni/kg bw. They found that, between two and 24 hr after injection, approximately 90% of serum ^{63}Ni was bound to proteins (e.g., albumin) with molecular weights greater than 10,000 and the remaining label was bound to small organic molecules such as short peptides and amino acids.

Chelation of Ni^{2+} by organic compounds has a significant effect on the cellular uptake, absorption, and distribution of Ni^{2+} (Sakar, 1984; Nierborer et al., 1984; Borg and Tjalve, 1988; Hopfer et al., 1987). Nierborer et al. (1984) studied cellular uptake of Ni^{2+} in human B-lymphoblasts, human erythrocytes and rabbit alveolar macrophages. They observed that addition of L-histidine or human serum albumin at physiological concentrations to the cell cultures reduced Ni^{2+} uptake by up to 70%. The concentration

of Ni^{2+} used in the study was 7 x 10^{-8} M (4.1 µg/L); it was comparable to serum nickel levels observed in workers occupationally exposed to nickel.

Rezuke et al. (1987) measured nickel concentrations in human postmortem samples in seven to 10 adults. In decreasing order the mean and range in µg Ni/kg dry weight in the tissue specimens were: lung 173 (71-371); thyroid 141 (41-240); adrenal 132 (53-241); kidney 62 (19-171); heart 54 (10-110); liver 50 (11-102); brain 44 (20-65); spleen 37 (9-95); and pancreas 34 (7-71). In five specimens of bile, nickel concentrations averaged 2.3 ± 0.8 µg/L (range 1.5-3.3 µg/L). These values differ markedly from the distribution of Ni in the rat noted in Table 3B above. The relatively high Ni burden in the human lung and low burden in the human kidney may indicate significantly more inhalation exposure in humans.

Nickel has been shown to cross the human placenta; it has been found in both fetal tissue (Schroeder et al., 1962) and the umbilical cord blood serum (McNeely et al., 1971). Similar findings have been reported in animal studies. Szakmary et al. (1995) administered a single gavage dose of 5.4, 11.3, or 22.6 mg Ni/kg bw as nickel chloride to pregnant rats. Twenty-four hours after exposure, nickel levels in fetal blood were raised from 10.6 (control) to 14.5, 65.5, and 70.5 µg/L for the low, medium, and high dose groups, respectively. Jacobsen et al. (1978) observed that when pregnant mice were given a single i.p. injection of ^{63}Ni chloride (0.14 mg/kg bw) on day 18 of gestation, passage of ^{63}Ni from mother to fetus was rapid and concentrations in fetal tissues were generally higher than those in the dam.

The distribution of nickel in pregnant and lactating rats following its injection has been studied by a number of authors (Dostal et al., 1989; Mas et al., 1986; Sunderman et al., 1978). Half-lives of nickel in whole blood following i.p. treatment of pregnant and non-pregnant rats were similar (3.6–3.8 hours), while the half-life for nickel in fetal blood was 6.3 hours following treatment on gestation days 12 or 19 (Mas et al., 1986). Intramuscular injection of nickel chloride (12 mg Ni/ kg/day) into pregnant and non-pregnant rats resulted in a greater accumulation of nickel in the pituitary of pregnant rats (Sunderman et al. 1978).

Tallkvist et al. (1998) evaluated the olfactory transport and subcellular distribution of $^{63}Ni^{2+}$ solution instilled intra-nasally in rats (4 µg/nostril). Cellular fractionation was conducted at one day, one week and three weeks after exposure. Of the $^{63}Ni^{2+}$ present in the olfactory epithelium, 60% to 70% was present in the supernatant, whereas in the olfactory bulb and the basal hemisphere about 70% - 80% of the nickel was bound to particulate cellular constituents. Gel filtration of the cytosol indicated that the $^{63}Ni^{2+}$ eluted with a molecular weight of about 250, identical to that obtained with histidine. Also, in olfactory tissues $^{63}Ni^{2+}$ was partly present in the cytosol associated with a 25,000 molecular weight component. The authors conclude that: 1) nickel is transported in the primary olfactory neurons via slow axonal transport; (2) the metal is bound to both soluble and particulate cytosolic constituents; and (3) the metal also shows this subcellular distribution in other parts of the olfactory system. The authors also note that

neuronal transport of nickel was about 20 times slower than cadmium ($^{109}Cd^{2+}$) or manganese ($^{54}Mn^{2+}$) studied earlier.

Schwerdtle and Hartwig (2006) evaluated the subcellular distribution of $NiCl_2$ and black NiO in human lung A549 cells exposed for 20 and 24 hr, respectively. Cells treated with $NiCl_2$ at 0, 50, 100, 250, or 500 µM exhibited dose-dependent uptake of Ni into the cytoplasm and nuclei. Intracellular Ni concentrations in cytoplasm were about 10, 20, 50, 275, and 550 µM, respectively. Concentrations in the nuclei were much lower at about 5, 10, 15, 40, and 110 µM, respectively. Cells treated with black NiO at 0, 0.2, 0.5, 1.0, and 2.0 µg NiO/cm^2 showed a similar pattern of intracellular distribution with greater relative concentrations in the nuclei. For cytoplasmic distribution the Ni concentrations were about 5, 110, 150, 240, and 450 µM, respectively. For nuclear distribution the Ni concentrations were about 2, 60, 70, 125, and 230 µM, respectively. The authors concluded that particulate Ni(II) exhibits greater toxicity due to its longer retention times rather than a different MOA which still involves Ni(II) ions as the direct or indirect genotoxicant.

4.3 Excretion

Nickel burden in humans does not increase with age. A majority of nickel absorbed from environmental media and diet is rapidly excreted via the urine. Solomons et al. (1982) found that nickel in water was quickly absorbed and excreted by humans; they estimated a biological half-life of about eight hours. Hogetveit et al. (1978) reported that elevated levels of nickel were detected in urine samples collected from workers exposed to soluble or insoluble nickel through inhalation.

The kinetics of nickel elimination in humans and animals appear to be similar. Onkelinx et al. (1973) injected nickel chloride i.v. to rats and rabbits and followed the nickel in plasma over time. Elimination profiles were similar in both species with early and later phases of elimination from plasma exhibiting first-order kinetics with half-lives of 6 and 50 hr for rats and 8 and 83 hr for rabbits, respectively.

Sweat and milk are also possible excretion routes for absorbed nickel in humans. Hohnadel et al. (1973) observed that, in sauna bathers, the mean concentrations of nickel in the sweat from healthy men and women were significantly higher than the mean concentrations in urine. Several studies have demonstrated that excretion of nickel in human milk is quite low and should be considered a minor route of excretion in lactating women (Feeley et al., 1983; Mingorance and Lachica, 1985). Casey and Neville (1987) reported a mean nickel concentration of 1.2 ± 0.4 µg/L (N = 46) in human milk samples from 13 women during the first month of lactation with an average estimated daily infant intake of 0.8 µg Ni. Krachler et al. (2000) measured trace elements in 27 human milk samples and found a median nickel concentration of 0.79 µg/L (range < 0.13-6.35 µg/L).

Graham et al. (1978) measured the clearance of $NiCl_2$ aerosol in mice exposed to 644 µg Ni/m^3 for two hours. Immediately following exposure and at 24 hr intervals thereafter the mice were sacrificed, their lungs and spleens were removed and weighed, and nickel concentrations were determined by atomic absorption spectroscopy. Clearance of nickel

from the lung followed first-order kinetics with a fitted curve of Y = 7.569exp (-0.291t), where Y is µg Ni/g dry weight lung and t is days post exposure. The spleen did not exhibit a significant uptake of nickel following exposure.

Koizumi et al. (2004) measured the urinary excretion of nickel in rats by inductively coupled plasma argon emission spectroscopy (ICPAES). Male Wistar rats received single oral doses of 0, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 10.0, 20.0, and 50.0 mg $Ni(NO_3)_2 \cdot 6H_2O$/kg bw. Five animals were used for analysis at each dose level. The 24-hr urinary excretion of nickel was observed to fit the relation $Y = 62.68X^{0.8527}$, R = 0.9488, where Y is the excreted Ni in µg and X is the oral dose in mg/kg bw. The proportion of total nickel elimination decreased from 25% at 0.01 mg/kg to about 5% at 0.1 mg/kg and higher doses. Urological analysis of markers of renal toxicity, N-acetyl-β-D-glucosamine (NAG), β_2-microglobulin, urine albumin, and urine protein, showed no indication of toxicity at any dose level used.

Dostal et al. (1989) showed that milk is an excretion pathway of nickel in rodents. Daily subcutaneous injections of lactating rats with 3 or 6 mg Ni/kg bw for four days raised nickel levels in milk from < 2 µg/L to 513 ± 54 and 1030 ± 66 µg/L, respectively. They also showed that nickel treatment significantly changed the composition of milk by increasing the milk solids (42%) and lipids (110%) and decreasing milk protein (29%) and lactose (61%).

Oyabu et al. (2007) studied the biopersistence of inhaled NiO nanoparticles in the rat lung. Thirty male Wistar rats were exposed to NiO nanoparticles (geometric mean diameter = 139±12 nm, average exposure concentration = $1.0 \pm 0.5 \times 10^5$ particles/m^3) for six hr/day for four weeks. At four days and one and three months after inhalation a group of 10 rats was sacrificed and the NiO particles deposited in the lung determined by chemical analysis. The retained Ni nanoparticle content of the lung decreased exponentially with a calculated half time of 62 days.

Oliveira et al. (2000) studied urinary nickel excretion in 10 workers from a galvanizing plant using $NiSO_4$ and 10 control subjects. Personal air monitors were used with 0.8 µm filters (OSHA method). No other particle size information was provided. Nickel airborne levels varied between 2.8 and 116.7 µg/m^3. Pre- and post-shift urinary Ni levels were taken on five consecutive workdays. Post-shift values ranged from 4.5 to 43.2 µg Ni/g creatinine. A significant correlation was observed between urinary and airborne nickel ($r = 0.96$, $P \leq 0.001$) with the relation urinary Ni (µg/g creatinine) = 6.00 + 0.43(airborne Ni, µg/m^3). No differences were observed with respect to different workdays.

Yokota et al. (2007) studied the urinary elimination of nickel and cobalt in relation to airborne exposures in a battery plant. The workers were exposed to nickel hydroxide, metallic cobalt, and cobalt oxyhydroxide. Nickel in the air was several fold higher than cobalt and positively correlated ($r^2 = 0.958$). Cobalt in air and post-shift urine gave a regression equation of $Co\ (\mu g/L)_{urine} = 15.8 + 243.8\ Co\ (mg/m^3)_{air}$ with a poor correlation coefficient (r = 0.491). No correlation was found between Ni in air and post-shift urine

[Ni $(\mu g/L)_{urine}$ = -17.3 + 7.33 Ni $(mg/m^3)_{air}$, r = 0.272, P = 0.15]. The authors note that the workers were using respiratory protection which presumably reduced inhalation exposure to $Ni(OH)_2$. They also note discrepancy with treatment of Ni inhalation by the DFG (Deutsche Forschungsgemeinschaft, 2005) which gives the relations for airborne nickel exposure and urinary nickel for water-soluble and water-insoluble compounds. For soluble nickel compounds including the hydroxide, acetate, chloride, sulfate and similar salts they give Ni $(\mu g/L)_{urine} = 10 + 600Ni(mg/m^3)_{air}$. For insoluble nickel compounds including the metal, oxide, carbonate, sulfide, and sulfidic ores they give Ni $(\mu g/L)_{urine} = 7.5 + 75Ni(mg/m^3)_{air}$. The authors argue that $Ni(OH)_2$ should be treated as an insoluble compound with respect to urinary excretion rather than a soluble one.

Afridi et al. (2006) measured metal content in biological samples from 56 production workers (PW) and 35 quality control workers (QCW) of a steel mill and 75 unexposed normal controls (all male, age range 25-55 yr). For nickel in scalp hair the PW showed the highest Ni concentration of 13.76 ± 4.48 µg Ni/g with QCW lower at 9.02 ± 2.64 µg Ni/g. These values were significantly higher than the non-occupationally exposed controls at 5.25 ± 1.46 µg Ni/g hair ($P < 0.02$). Surprisingly the mean lead values were quite similar at 16.21, 10.33, and 6.84 µg Pb/g hair, respectively. Urine concentrations were also measured and showed lesser, but also significant, differences i.e. 9.47, 7.62, and 6.31 µg Ni/L urine, respectively.

Ohashi et al. (2006) evaluated selected urinary metals in 1000 women in the general Japanese population. The geometric mean concentration for nickel was 2.1 µg Ni/L or 1.8 µg Ni/g creatinine. Unlike copper and manganese both nickel and cobalt showed no substantial age dependency for urinary excretion.

4.4 Physiological Models

4.4.1 Biokinetic Models

Onkelinx et al. (1973) conducted a kinetic analysis of $^{63}Ni^{2+}$ clearance in rats and rabbits following a single intravenous injection of $^{63}NiCl_2$. In both species $^{63}Ni^{2+}$ was rapidly cleared from plasma or serum during the first two days, and more slowly after two days. The blood elimination data was best described by the bi-exponential relations:

$$\text{Rats: } S = 226 \exp[-0.11t] + 0.57 \exp[-0.014t];$$

$$\text{Rabbits: } S = 1165 \exp[-0.092t] + 4.95 \exp[-0.0084t];$$

Where S is the plasma concentration of Ni^{2+} (µg/L) and t is the time after injection (hr). A two-compartment model derived from the data successfully predicted serum or plasma concentrations of Ni^{2+} in animals receiving continuous infusions or repeated daily injections of $^{63}NiCl_2$.

Sunderman et al. (1989) developed a model to predict nickel absorption, serum levels, and excretion following oral exposure to nickel in water and food. The model was developed based on two experiments in humans in which serum nickel levels and urinary

and fecal excretion of nickel were monitored for two days before and four days after eight subjects were given an oral dose of nickel as nickel sulfate (12, 18, or 50 µg Ni/kg bw) in water or in food. The data were then analyzed using a four-compartment toxicokinetic model consisting of Gut, Serum, Urine and Tissues. Two inputs of nickel, the single oral dose, in which uptake was considered to be a first-order process, and the baseline dietary ingestion of nickel, in which uptake was considered to be a pseudo-zero order process were used. Model parameters were determined for the model from the two experiments. No further model validation (i.e. with independent data) was described. A sample model code implemented in Berkeley Madonna software is given in the Appendix (A.1) for a single 50 µg Ni/kg bw dose in water.

Uthus (1999) proposed a 16-compartment biokinetic model to describe the uptake and metabolism of orally administered $^{63}NiCl_2$. The compartments were either in groups representing the GI tract, Blood, Liver or Body, or individual for Urine and Feces. Transfer of Ni mass between compartments was governed by first order rate constants. Oral dosing of female Sprague-Dawley rats with 0.84 µg ^{63}Ni (10.7 µCi) resulted in seven day cumulative urinary and fecal excretions of 2.46% and 97.5% of dose, respectively. For liver, peak ^{63}Ni radiolabel occurred within 30 min of dosing and reached 0.09% of dose. Peak radiolabel in kidney was 0.04% of dose and in bone 0.001% of dose. The model predicts 2.54% and 96.4% of dose excretions for urine and feces, respectively. Retention of Ni in grouped organs was predicted to amount to 0.34% seven days after dosing. Model code for a single oral dose is provided in the Appendix.

Franks et al. (2008) describe a mathematical model of the in vitro keratinocytes response to chromium or nickel exposure. The model tracks the interaction between metal ions (in both intra- and extra-cellular states) and their effect on the viability of keratinocytes and the release of the pro-inflammatory cytokine interleukin-1α (IL-1ά). The model is intended to describe a monolayer of freshly isolated keratinocytes, which has been grown to confluence and dosed with media containing, e.g., 0.01 to 10,000 µM $NiCl_2$ for 24 hours. The metal ion is assumed to be in equilibrium between extracellular concentration (Ac) and intracellular concentration (Ai), with the latter inducing the cytokine response. The volume fraction of keratinocytes is (n) and the amount of metal associated with the cell is given by (nAi). The volume fraction of keratinocytes in the system is described by $dn/dt = - Kdn$; where $Kd = \delta niAi + \delta n$. This accounts for death due to the toxic effects of the intracellular ion (δniAi) and the net birth and natural death of cells (δn). Control experiments indicated that 80% of cells were still alive after 24 hr, indicating that $\delta n > 0$. The model assumes: (1) an exchange between extra- and intracellular metal ions; (2) cell death releases metal ions to the extracellular region; and (3) partitioning between extra- and intracellular states according to a partition coefficient (μ_n). The main equations for extra- and intracellular metal ions, respectively, are as follows:

$$d/dt((1 - n)Ac) = -knn(\mu_n Ac - Ai) + KdnAi;$$

$$d/dt(nAi) = knn(\mu_n Ac - Ai) - KdnAi.$$

Keratinocytes with metal bound to them release a variety of chemokines and cytokines, in particular IL-1ά, release of the latter is described in the model by:

$$d/dt((1 – n)c) = \beta_{cn}n + \beta_{ci}nAi - \delta c(1-n)c;$$

where βcn is the rate of cytokine release by unaffected cells, βci is the rate of cytokine release by affected cells, δc is the rate of natural decay of cytokines in the media, and c is the concentration of IL-1ά. In comparing model predictions to experimental data for nickel the authors report no apparent relationship between nickel dose and IL-1ά release except a decrease at high nickel concentrations (> 100 μM). Good agreement between model predictions and existing experimental data was observed. An example implementation of the Franks et al. model in Berkeley Madonna code is given in the Appendix (A.2).

4.4.2 Physiologically-Based Pharmacokinetic (PBPK) Models

No PBPK models for nickel compounds were identified in the published literature. An example of what a nickel PBPK model might look like is given in the appendix (A.4). This example is based in part on the manganese rat PBPK model of Teeguarden et al. (2007). The model was adjusted for nickel using data from Ishimatsu et al. (1995), Benson et al. (1994) and Tanaka et al. (1985). The model represents six perfused tissues: upper and lower respiratory tracts, bone, liver, kidneys, and muscle. Each of these tissues has a shallow tissue pool in rapid equilibrium with blood and a deep tissue store connected to the shallow tissue by transfer rate constants. Exchange of nickel between the shallow tissue pools and venous blood is controlled by tissue/blood partition coefficients (Ishimatsu et al., 1995). Absorption of airborne nickel oxide includes transport of deposited nickel into shallow tissue pools and mechanical removal from respiratory surfaces to the gastro-intestinal tract. The model includes fecal, urinary and biliary excretion of absorbed or ingested nickel. Comparisons of model predictions with observed data of Tanaka et al. (1985) for prolonged exposures to NiO aerosol were good for lung tissue Ni concentrations at high and low exposure concentrations and for liver and kidney concentrations at high exposure concentration (App. A.4).

Hack et al. (2007) describe a physiological model of the intracellular dosimetry of inhaled nickel. The model consists of seven intracellular compartments of the tracheobronchial epithelial cell: Cytoplasm, Cytoplasmic proteins, Vacuolar Particles, Perinuclear Cytoplasm, Perinuclear Cytoplasmic Proteins, Nucleus, and Nuclear Proteins. Extracellular compartments consist of Surface Particles, GI Tract, Ionic Ni in Mucus, and Venous Blood. The model accepts the deposited dose into the mucous layer following inhalation of nickel particles or aerosols.

Particulate nickel compounds are either cleared from the mucous layer by mucociliary action, dissolved into Ni^{2+} ions, or taken up by the cells. Phagocytosis of nickel particles, such as Ni_3S_2 or crystalline NiS, results in the formation of a vacuole in which nickel particles are encased and ultimately dissolved. Extracellular dissolution of soluble nickel compounds results in the release of ionic nickel, which enters the cell via divalent ion transport systems (e.g., magnesium). Both influx and efflux of nickel ions are described

by saturable Michaelis-Menten kinetics. Once in the cytoplasm nickel ions may bind with cytosolic proteins or diffuse through the cytoplasm to the perinuclear cytoplasm. Once there, nickel ions may bind reversibly to perinuclear proteins, enter the nucleus and bind to nuclear proteins. Model processes are mostly modeled with first order rate constants for forward and reverse directions. An exception is the Michaelis-Menten kinetics for influx and efflux of Ni from mucous to cytoplasm to venous blood. In this respect the Hack et al. model resembles a biokinetic model. Model parameters were based mostly on published values. An example of this model implemented in Berkeley Madonna code is given in Appendix (A.3).

The model for uptake of $NiCl_2$ by cultured pneumocytes predicted steady state concentrations better than the rate of uptake where the model underpredicted intracellular levels in the first half hour after exposure (data of Saito and Menzel, 1986). Model comparisons with the data of Costa et al. (1981) gave good observed/predicted ratios (O/P) of 1.57 to 0.94 for Ni_3S_2 in the nucleus (nmol/mg protein), 0.65 for $NiCl_2$ in the whole cell, 0.3 for $NiCl_2$ in the cytoplasm, and 0.5 for $NiCl_2$ in the nucleus (all nmol/mg protein). With the data of Abbracchio et al. (1982) agreement was more variable for O/P: $NiCl_2$ in the nucleus, 2.5 to 5.7; $NiCl_2$ in cytoplasm, 0.18; crystalline NiS in the nucleus 0.96 to 3.5; crystalline NiS in the particulate fraction 1.02; crystalline NiS in the cytoplasmic fraction 1.10.

4.4.3 Lung Deposition-Clearance Models

Hsieh et al. (1999a) proposed a dosimetric model of nickel deposition and clearance from the rat lung. The model was developed using lung burden data from the National Toxicology Program (NTP) studies of nickel sulfate (NTP, 1996c), nickel subsulfide (NTP, 1996b), and nickel oxide (NTP, 1996a) and earlier models (Yu and Xu, 1986). The model consists of a single alveolar compartment. Deposited particles are removed from the lung by two principal mechanisms: (1) mechanical clearance via mucociliary transport; and (2) clearance by dissolution. For moderately soluble Ni_3S_2 particles both mechanisms are operable. The lung burden buildup in the alveolar region of the rat lung is described by the following equations:

$$dMi/dt = ri - \lambda iMi \qquad (1);$$

$$ri = Ci \times \eta\, MV \qquad (2);$$

$$\lambda i = ai \exp[-bi\{m_s/m_{s0})^{ci}] \qquad (3);$$

where M is the mass burden, i indicates the particular nickel compound, r is the deposition rate, λi is the total alveolar clearance rate coefficient, η is the alveolar deposition fraction, Ci is the air concentration, MV is the minute ventilation, ai, bi, ci are compound specific clearance rate coefficient constants, m_s = M/S in which M is the lung mass burden and S is the total alveolar surface area ($m_s = 5.38 \times 10^3$ cm^2 for rats), and m_{s0} = 1 mg/cm^2 is the dimensional constant introduced to normalize m_s. For $NiSO_4$, the a, b, c parameter values were 10.285, 17.16, and 0.105, respectively. For Ni_3S_2, the a, b, c

parameter values were 0.00768, -20.135, and 0.266, respectively. For NiO, the a, b, c parameter values were 0.0075, 300, and 0.95, respectively.

Hsieh et al. (1999b) modified the rat model to develop a model of deposition and clearance of nickel in humans. Deposition rates were calculated for six scenarios: nose-breathing at rest, nose-breathing at light work, nose breathing at moderate work, mouth breathing at rest, mouth breathing at light work, and mouth breathing at moderate work. The clearance rate coefficient constants for humans were modified from the rat values. For nickel oxide, clearance rate coefficient constant *a* was estimated to be 1/7.6 times the rat value; constants *b* and *c* were assumed to be the same as rats. For nickel subsulfide, clearance is due to mechanical transport and dissolution; the clearance rate coefficient constant *a* was estimated to be the sum of the clearance rate coefficient constant *a* for insoluble nickel (nickel oxide) and the difference between the clearance coefficient constant for nickel oxide and for nickel subsulfide for rats. For nickel sulfate, clearance rate coefficient constants in humans were assumed to be the same as in rats.

Hsieh et al. (1999c) developed a model for deposition, clearance and retention kinetics in the respiratory tract for inhaled nickel compounds in the mouse. The nickel compounds studied were NiO (green), Ni_3S_2, and $NiSO_4 \cdot 6H_2O$. The approach and equations for alveolar deposition and clearance are similar to those given above for the rat (Hsieh et al., 1999a). In this case the compound specific clearance coefficients a, b, c were: NiO, 0.0085, 180, 0.95; Ni_3S_2, 0.011, -9.293, 0.266; and $NiSO_4$, 10.285, 15.78, 0.105, respectively. The model predictions were compared with experimental data for the normalized lung burden metric (Ni-lung burden/g lung/unit concentration) and the calculated results did not always show good agreement. Because of lower deposition rates and faster clearance rates, mice have lower lung burdens than rats when exposed to the same concentrations of NiO or $NiSO_4$ particles. For Ni_3S_2, the lung burden/gram of lung in mice can be lower or higher than in rats depending upon exposure concentration and duration.

The Yu et al. (2001) modification of the model was used to predict lung burdens in nickel refinery workers and comparison with measured lung Ni burdens in deceased refinery workers showed good agreement between predicted and measured values. The model treats the alveolar region of the human lung as a single compartment. The kinetic expressions governing the change in mass with time in this compartment for NiO, Ni_3S_2 and $NiSO_4$ are as follows:

$$dM_{NiO}/dt = r_{NiO} - \lambda_{NiO} M_{NiO};$$

$$dM_{Ni3S2}/dt = r_{Ni3S2} - \lambda_{Ni3S2} M_{Ni3S2};$$

$$dM_{NiSO4}/dt = r_{NiSO4} - \lambda_{NiSO4} M_{NiSO4};$$

where M is the mass burden, r is the deposition rate and λ is the total alveolar clearance rate coefficient over all clearance pathways. For a given concentration, r in the above

expressions is equal to concentration x alveolar deposition fraction (η) x minute ventilation (MV). The clearance rate coefficients are based on extrapolation from rat data e.g.

$$\lambda_{NiO} = 0.00099\exp[-300(V/V_{AM})^{0.95}] \text{ (/day);}$$

where V is the total volume of Ni compounds retained in the lung and $V_{AM} = 1.75 \times 10^4$ mm^3 is the total alveolar macrophage volume in humans. When the dosimetry model is applied to worker exposure, three additional factors are incorporated in the model: inhalability, mixed breathing mode, and clearance rate coefficient of a Ni compound mixture. The inhalability expression is based on the recommendation of the International Commission on Radiological Protection (ICRP ,1994):

$$\eta_{inhalability} = 1\text{-}0.5*(1\text{-}(7.6 \times 10^{-4}\ d_a^{2.8} +1)\text{-}1 + 1.0 \times 10^{-5}\ U^{275} \exp(0.055d_a);$$

where d_a is the aerodynamic diameter of the particle in μm and U is the wind speed in m/s. Deposition rates are calculated for three different ventilations: at rest, light work, and moderate work.

This modified dosimetry model was applied to the data on lung Ni burden for 39 workers reported by Andersen and Svenes (1989). Since particle sizes were not measured in the study values from the same facility measured by Vincent (1996) were used. Particle sizes ranged from 42 to62 μm MMAD for roasting and smelting and 1.4 to 51 μm MMAD for electrowinning work areas. These values are much greater than the 2 to 3 μm MMAD used in the chronic rat inhalation study. The correspondence of observed vs. predicted lung burdens for the two work areas are presented by the authors (their Figs. 1 and 2) but no statistical correlations were provided. Nevertheless several points fall on or close to the 1:1 correlation line generally supporting their claim of good agreement.

5. Acute Toxicity to Humans

Soluble nickel compounds appear to be the greatest concern for acute health effects. The soluble forms of nickel are absorbed as Ni^{2+} (Coogan *et al*., 1989). Divalent nickel competes with copper for binding to serum albumin and is systemically transported in this way (Sunderman, 1986). The kidneys, lungs, and placenta are the principal organs for systemic accumulation of nickel (Sunderman, 1986). In contrast to the long half-life of the insoluble forms of nickel in the nasal mucosa, the elimination half-life of Ni^{2+} in the plasma is 1-2 days in mice (Nieboer *et al*., 1988).

A two-year-old child died after accidentally ingesting an oral dose of approximately 570 mg/kg bw of nickel sulfate (Daldrup et al., 1983). Cardiac arrest occurred four hours after the ingestion, and the child died eight hours after the accident. Webster (1980, cited in Norseth, 1984) reported nickel intoxication in a group of 23 dialysis patients. The source of nickel was plated stainless steel in a water heater tank. The concentration of nickel was approximately 250 μg/L in the dialysate. This level was much higher than those found in five other dialysis units (average 3.6 μg/L, range 2.5 to 4.5 μg/L).

Symptoms observed included nausea, weakness, vomiting, headache and palpitations. Remission was relatively rapid occurring in three to 13 hours after cessation of dialysis. Sunderman et al. (1988) report on an episode of 32 workers in an electroplating plant accidentally drinking water containing $NiSO_4$ and $NiCl_2$ with a concentration of 1.63 g Ni/L. Twenty workers experienced nausea, vomiting, abdominal discomfort, diarrhea, giddiness, lassitude, headache, cough and shortness of breath, which lasted for a few hours to several days. Nickel intakes were estimated at between 0.5 and 2.5 g. Serum concentrations ranged from 13 to 1340 μg Ni/L and urine concentrations from 0.15 to 12 mg Ni/g creatinine. Elimination half times ranged from 27 hr with induced diuresis to 60 hr in non-induced subjects.

Nickel fumes from high nickel alloy welding (mean concentration = 440 μg Ni/m³, range = 70-1,100 μg Ni/m³) caused complaints of upper respiratory irritation and headache in welders exposed for 4 weeks (Akesson and Skerfving, 1985).

A group of seven metal plating workers with occupational asthma was evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla et al., 1985). Three of the asthmatics tested positive for the presence of nickel-specific immunoglobulin E (IgE) antibodies. Positive reactions to skin testing with nickel were found in three of the asthmatic workers who also had dermatitis. Six out of the seven asthmatics exhibited significantly decreased forced expiratory volume (reduction in FEV_1 > 15%) when exposed to 0.3 mg/m³ nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV_1.

Exposure to nickel in occupational settings causes dermatitis and asthma in some individuals with repeated exposures (Davies, 1986). The nickel ion, bound to proteins in the dermis, acts as an antigen eliciting a type IV (delayed type) hypersensitivity response. This response, mediated by T-lymphocytes, causes dermal hypersensitivity. This hypersensitivity can be diagnosed by patch testing (Menne and Maibach, 1989).

Inhalation of nickel dust has been associated with increased incidence of pulmonary fibrosis. A potential mechanism is via inactivation of the pulmonary fibrinolytic cascade (Andrew and Barchowsky, 2000). Andrew et al. (2001) studied the effect of nickel subsulfide on activator protein-1 (AP-1) induction of plasminogen activator inhibitor-1 (PAI-1). Addition of 2.34 μg Ni/cm^2 Ni_3S_2 to BEAS-2B human airway epithelial cells stimulated intracellular oxidation, induced c-Jun and c-Fos mRNA levels, increased phospho- and total c-Jun levels, and increased PAI-1 mRNA levels over a 24-hr treatment period. No cytotoxicity was observed with nickel treatment. Pretreatment with the antioxidants N-acetyl-L-cysteine and ascorbic acid blocked the nickel-induced increases in reactive oxygen species (ROS) but did not affect the nickel induction of PAI-1. The results indicate that the potential effect of nickel on fibrinolytic activity is independent of its participation in redox cycling.

Barchowsky et al. (2002) exposed BEAS-2B human airway epithelial cells in culture to non-cytotoxic levels (based on cell survival assays) of Ni_3S_2 and observed increased expression of the inflammatory cytokine interleukin-8 (IL-8). Confluent cells were

treated with 2.34 µg Ni/cm^2 nickel subsulfide for 24 or 48 hr. After 48 hr there was a statistically significant increase in IL-8 protein in the culture medium compared to the control (ca. 2.3 vs. 0.9 ng/mL, $P < 0.001$, their Fig.1). No increase was seen after 24 hr. IL-8 mRNA levels preceded the increase in IL-8 protein. Transient exposure to soluble nickel sulfate failed to increase IL-8 mRNA. Further study revealed that nickel induced IL-8 transcription through a novel pathway that requires both AP-1 and non-traditional transcription factors, Fos and cJun. The authors note that the protracted course of particulate nickel-stimulated IL-8 production observed in the study contrasts with the immediate IL-8 induction in response to cytokines, hypoxia, and many inhaled toxicants. Thus the study indicates "particulate Ni_3S_2 activates specific signaling cascades following uptake by pulmonary epithelial cells. These activated cascades stimulate parallel pathways for inducing transcription of both inflammatory and profibrotic genes."

Predisposing Conditions for Nickel Toxicity

Medical: Asthmatics or atopic individuals may be especially at risk for developing nickel-induced asthma (Cirla *et al*., 1984). Cigarette smokers may receive greater nickel exposure, since cigarette smoke contains nickel (Reprotext, 1999). Additionally, a review of the literature on nickel toxicity showed that Ni^{2+} causes vasoconstriction in animals and humans, which may potentiate the effects of a primary ischemic lesion in the cardiovascular system (U.S.EPA, 1985).

Chemical: In rats, rabbits, and dogs, one mg/kg nickel chloride antagonizes the cardiac arrhythmia induced by digoxin by competing with calcium at cardiac membrane sites (Prasad *et al*., 1980). The implications of this effect for persons with congestive heart failure have not been investigated.

6. Acute Toxicity to Laboratory Animals

It has been shown that water-soluble nickel compounds are more acutely toxic than the less soluble compounds. The single dose oral LD_{50}'s in rats for less-soluble NiO and Ni_3S_2 were > 3000 mg Ni/kg bw, while the oral LD_{50}'s for the more soluble $NiSO_4$ and nickel acetate ranged from 39 to 141 mg Ni/kg bw in rats and mice (Mastromatteo, 1986; Haro et al., 1968). Soluble nickel compounds appear to be more toxic by intraperitoneal (i.p.) injection than by intramuscular (i.m.) or subcutaneous (s.c.) injections. Acute LD_{50} values for $NiCl_2$ in rats were 5 mg Ni/kg bw by i.p. injection, 23 mg Ni/kg bw by i.m. injection, and 25 mg Ni/kg bw by s.c. injection (Knight et al., 1991).

Subcutaneous injections of 10 mg/kg nickel chloride have been shown to increase prolactin secretion in rats one day following administration (Clemons and Garcia, 1981). However, an earlier study showed that prolactin secretion in rats is specifically inhibited for 30 minutes following intravenous exposure to 100 µg Ni^{2+} as $NiCl_2$ (LaBella *et al*., 1973). Donskoy et al. (1986) found that s.c. injection of 125 to750 µmol Ni/kg to male Fisher 344 rats resulted in acute hepatic toxicity within 24 hr as evidenced by enhanced lipid peroxidation, microvesicular steatosis, and increased serum aspartate

aminotransferase (AST) and alanine aminotransferase (ALT). The latter serum enzymes were significantly increased about two-fold by the low dose of 125 µmol Ni/kg compared to control animals ($P < 0.05$, $N = 14$).

Subacute (12-day) inhalation exposures (5 days/week, 6 hours/day) of 10 mice to nickel, as 10 mg Ni_3S_2/m^3, caused 100% mortality (Benson *et al*., 1987). Two of 10 rats also died from this exposure. Although no effect was seen on natural killer cell activity in these animals, lesions in the nasal and lung epithelium and in bronchial lymph node were observed. Pathology revealed emphysematous changes in the lungs of rats exposed to 5 or 10 mg Ni_3S_2/m^3, and fibrosis in mice exposed to 5 mg Ni_3S_2/m^3. Atrophy of lymphoid tissues, including spleen, thymus, and bronchial lymph nodes, was observed in mice and rats exposed to 5 or 10 mg Ni_3S_2/m^3.

Studies by Graham et al. (1975, 1978) indicate that the immune system is a sensitive target for acute nickel toxicity showing inhibition of antibody production against sheep erythrocytes. These authors used a hemolytic plaque technique to determine the number of specific antibody-producing spleen cells. Six-week old SPF female Swiss mice (14-29 per group) were exposed by inhalation to 0, 100, 250, 375, or 490 µg Ni/m^3 as $NiCl_2$ (99% of particles were ≤ 3µm in diameter, exposure values were estimated from their Fig. 3) for two hours. The exposed animals showed a significant decrease in splenic antibody-forming cells following a challenge with a T-lymphocyte dependent antigen (Graham *et al*., 1978). A linear dose-response was observed with a negative linear regression of $Y = -34.9 - 0.347X$, where Y is the number of hemolytic plaques formed/10^6 spleen cells and X is the exposure concentration in µg Ni/m^3. The results indicate a LOAEL of 250 µg Ni/m^3 and a potential NOAEL of 100 µg Ni/m^3. Unfortunately this study is short on details and the NOAEL is not considered as reliable as the LOAEL (no control values are given). We analyzed the data in the Graham et al. (1978, Figure 3) with a continuous benchmark dose approach. The extrapolated background from their Fig. 3 is approximately -40 plaques/10^6 cells. Using a criterion of a -100 plaques/10^6 cells as a significant effect (more than doubling the background), we obtained a good fit to a linear model ($P = 0.95$) with a BMD for a 100 plaque loss of 284 µg Ni/m^3 and a BMDL of 164.6 µg Ni/m^3 (Fig. 1). The latter value is treated as an adjusted LOAEL in the derivation of the 8-hour REL (Section 10.2).

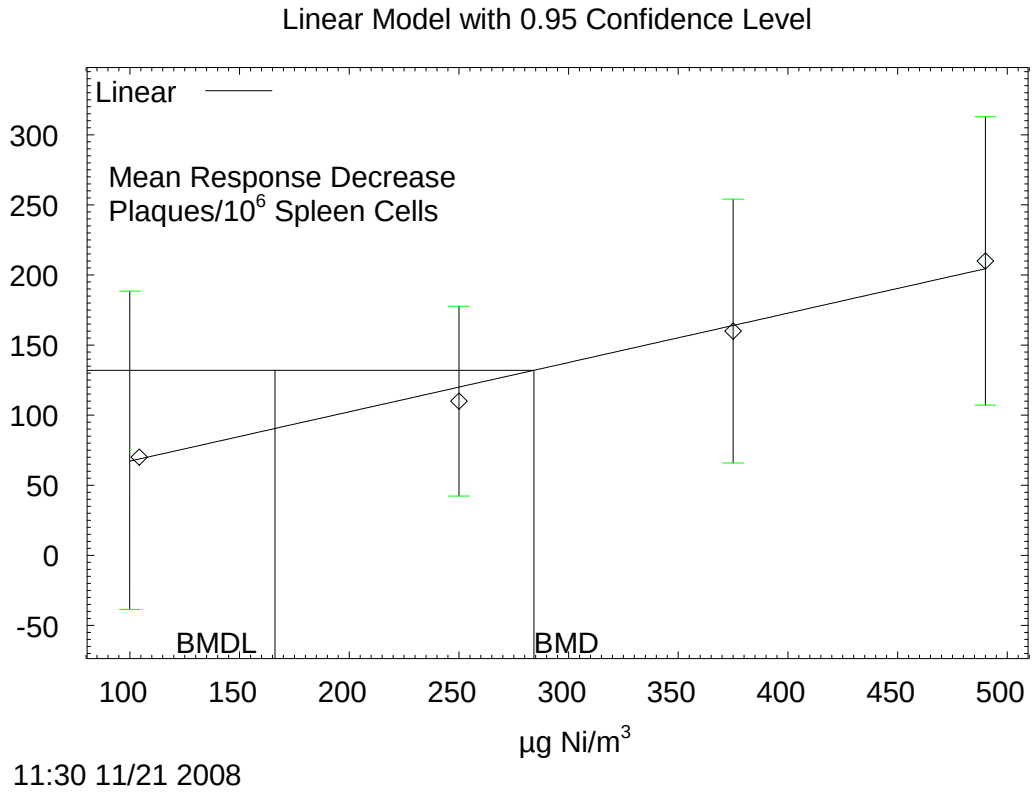

Figure 1. Continuous Benchmark Dose Analysis of Decrease in Plaques/10^6 Spleen Cells vs. µg Ni/m^3, 2 Hours Exposure of Female Swiss Mice. BMD and BMDL are for a 100 Plaque Decrease (data from Graham et al. 1978, Fig.3).

A similar suppression in antibody-forming cells was seen in mice (10-12/dose group) exposed intramuscularly to 0, 3.09, 6.17, 9.25, or 12.34 µg Ni/g body weight as $NiCl_2$ or $NiSO_4$ (Graham *et al*., 1975, 1978). Statistically significant decreases in plaque production ($P < 0.05$ vs. control by William's test) were seen at 9.25 µg Ni/m^3 with $NiCl_2$ and at 3.09 µg Ni/m^3 with $NiSO_4$ (Graham et al., 1975). Similar exposures with NiO showed no decreases at any dose. A linear dose-response was given for $NiCl_2$ of Y -= 2.64 – 0.028X, where Y is the log10 of plaques/10^6 cells and X is the i.m. dose of µg Ni/g bw.

Condevaux et al. (2001) compared the effects of morphine and nickel chloride on natural killer (NK) cell activity in vitro in rats and in the cynomolgus monkey. The NK cells were exposed to either $NiCl_2$ at 0, 1, 10, or 100 µg/mL or morphine at 0, 0.01, 1, or 1000 nM. There were statistically significant decreases in NK cell activity at the highest concentrations of nickel or morphine. The magnitudes of the decreases were greater in the monkey than in the rat, i.e. for $NiCl_2$ the decreases were 34.4-42.2% in monkey and 21.6-24.3% in rat. Morphine hydrochloride induced decreases of 59.1-68% in the monkey and 23.7-34.7% in the rat.

Haley *et al*. (1987) showed that male cynomolgus monkeys, exposed to intratracheal Ni_3S_2 at a delivered dose of 0.06 μmol Ni/g lung tissue, had impaired pulmonary macrophage phagocytic function and increased NK cell activity. Mice also exhibited impairment of pulmonary macrophage function in addition to decreases in antibody-forming spleen cells with inhalation exposure to Ni_3S_2 or NiO (Haley *et al*., 1990). Natural killer cell activity measured by splenic cytotoxic activity to tumor cells as well as by clearance of melanoma tumors *in vivo* was suppressed in two strains of mice exposed to intramuscular injections of 18.3 mg Ni/kg as $NiCl_2$ as compared to controls (Smialowicz *et al*., 1985).

A host-resistance study by Adkins *et al*. (1979) showed that mice (80-120 per group) exposed to inhaled soluble nickel for two hours in the form of $NiCl_2$ or $NiSO_4$ were significantly more susceptible to mortality from streptococcal bacterial infection. The concentrations of nickel that showed these effects were 499 μg Ni/m³ ($NiCl_2$) and 455 μg Ni/m³ ($NiSO_4$). No significant change in mortality was seen with exposure to 369 μg Ni/m³ as $NiCl_2$.

Nickel distributes preferentially to the lungs and kidneys following intratracheal administration of $NiCl_2$ to rats (Carvalho and Ziemer, 1982). The electrophilic Ni^{2+} ion is reported to be the causative agent of nephrotoxicity in rats; it binds to intracellular nucleophiles in kidney tissue such as guanine, adenine, and glutathione two hours following intraperitoneal exposure to 10 mg Ni/kg as $NiCO_3$ (Ciccarelli and Wetterhahn, 1984).

Toya et al. (1997) evaluated the effects of single and repeated intratracheal instillations of nickel fumes, Ni_2O_3 and NiO powders, all dispersed in saline and sonicated immediately prior to instillation, in the Sprague-Dawley rat. The LD_{50} of nickel fumes was estimated to be 38.2 mg/kg bw. Body weight gain was retarded by single doses of 13.0 mg Ni_2O_3/kg, 14.3 mg Ni fumes/kg, or 13.0 mg NiO/kg compared to controls. The lung lesions induced by a single nickel exposure were characterized by goblet cell hyperplasia, perivascular inflammatory cells and edema in the alveolar space. Nickel fumes and Ni_2O_3 produced goblet cell hyperplasia, focal granuloma, and inflammatory cells in the alveolar space but NiO did not produce lesions. Repeated instillations of nickel fumes (5.9 mg/kg-d for four days to one week) produced a secretion of proteinaceous materials in the alveolar space. The authors note that although the Ni fumes were composed of about 3% Ni_2O_3 and the remainder NiO, its toxicity was greater on a weight basis than Ni_2O_3 administered alone. They speculate that the difference in toxicity was due to the presence of ultrafine particles in the Ni fumes.

Serita et al. (1999) studied lesions formed in rat lungs after a single five hour exposure to ultrafine metallic nickel (Uf-Ni) with a 20 nm average particle diameter (MMAD = 1.3 μm, geometric standard deviation (gsd) = 1.54 μm). Sixty to 80 Wistar rats per dose group (sex unspecified) were exposed to 0.15 (Low), 1.14 (Medium), or 2.54 (High) mg Uf-Ni/$m^3$for five hours. Five animals /dose group were sacrificed at 0 hr and 1, 3, 7, 14, and 21 days post exposure. The Low group also had sacrifices at 28, 56, and 84 days post exposure. The toxicological findings included: (1) a significant increase in lung

weight in the Medium and High groups; (2) accumulation of foamy alveolar macrophages (AM) and debris of burst AM which may restrict pulmonary ventilation; (3) degenerated AM indicating alveolar lipoproteinosis which was aggravated for up to four weeks in the High group; and (4) acute calcification of the degenerated AM possibly related to a disruption of Ca^{2+} ion transport by solubilized Ni^{2+} ion. This study indicates a LOAEL of 1.14 mg Ni/m^3 and a NOAEL of 0.15 mg Ni/m^3 for a single five-hour exposure to metallic nickel. However, as the authors point out, if half of the amount of Ni deposited in the lung in the Low group were carried over to the next day, the amount of deposition after 30 days at 5hr/d would exceed the single exposure deposition for the High group. Therefore, it is difficult to accept 0.15 mg/m^3 as a true NOAEL applicable to repeated exposure scenarios.

Prows and Leikauf (2001) studied the genetic determinants underlying the susceptibility to acute nickel-induced lung injury in sensitive and resistant mouse strains. The mice were exposed 6 times over one year in an inhalation chamber continuously to air containing 152 ± 12 µg Ni/m^3 (0.2 µm MMAD) generated from 50 mM (10^{-3} M) $NiSO_4 \cdot 6H_2O$ (duration of individual exposures not given). Quantitative trait loci (QTL) analysis of 307 backcross mice generated from the sensitive A/J and resistant C57BL/6J mouse strains identified a significant linkage on chromosome 6 (designated *Aliq4*) and suggestive linkages on chromosomes 1 and 12. Analysis of phenotypic extreme responders to nickel-induced lung injury, including 55 most sensitive (survival times ≤ 66 hr) and 54 most resistant (survival times ≥ 112 hr) backcross mice, identified possible linkages on chromosomes 1, 6, 8, 9, and 12, which explained 62% of the genetic variance in the extreme phenotypic cohort. Comparing mean survival times of backcross mice with similar haplotypes gave an allelic combination of four QTLs that could account for the survival differences. The QTL intervals on chromosomes 6 and 12 were previously identified with ozone sensitivity. Candidate genes for chromosome 6 locus include *Tbxas1* (thromboxane A synthase 1), *Aqp1* (aquaporin-1), *Crhr2* (corticotropin releasing hormone receptor-2), *Sftpb* (surfactant-associated protein-B), *Pecam* (platelet/endothelial cell adhesion molecule), and *Tgfa* (TGF-α). The results suggest that relatively few genes might be important for irritant-induced acute lung injury.

In a subsequent study (Prows et al., 2003), cDNA microarray analysis was employed in the analysis of nickel sensitive (A/J) and resistant (C57BL/6J) mouse strains. The mice were exposed continuously to150 µg Ni/m^3 (MMAD = 0.22 µm, gsd = 1.85 µm) for 3, 8, 24, or 48 hr. Significant expression changes were identified in one or both strains for more than 100 known genes. The results indicated a temporal pattern of increased cell proliferation, extracellular matrix repair, hypoxia, and oxidative stress, followed by reduced surfactant proteins. Fifteen functional candidate genes were associated with expression ratio differences of two-fold or greater between strains for at least one exposure time. Of these two genes—metallothionein-1 (*Mt1*) on chromosome 8 and SP-B (*Sftpb*) on chromosome 6—map to QTL intervals linked to nickel-induced acute lung injury survival.

Ishihara et al. (2002) studied inflammatory responses and mucus secretion in rats with acute bronchiolitis induced by nickel chloride inhalation. Male Wistar-jcl strain SPF rats

at age 10 weeks were exposed (5 animals/group) via whole body to aerosols of nickel chloride with an ultrasonic nebulizer 5 hours/day for 5 days. The average concentrations of the aerosols were 0.85 mg Ni/m^3 in day one and 0.24 mg Ni/m^3 during days two to five. Following exposure the animals were given clean air on days six to eight prior to sacrifice. The nickel aerosols had a MMAD of 1.8 µm with a gsd of 1.6 µm. The measured inflammatory biomarkers were total protein concentration, total elastolytic activity, α1-antitrypsin, and β-glucuronidase activity. Sialic acid and fucose were measured as mucus components. Also measured were soluble L-selectin, cytokine-induced neutrophil chemoattractant (CINC) and growth-regulated gene products (GRO). Total protein concentrations, total elastolytic activity, trypsin inhibitory capacity, β-glucuronidase, fucose, and sialic acid in bronchoalveolar lavage fluid (BALF) were significantly greater than control ($P < 0.05$ vs. control, $N = 5$) at day 3 to day 8 time points following nickel exposure. CINC/GRO and soluble L-selectin were significantly increased at days 3-6 and days 5-6, respectively. The extent of lung tissue injury was scored by histopathological observations. There was no exfoliation of the airway epithelium found on exposure day five when bronchiolitis developed. The data indicate that nickel chloride inhalation caused an acute inflammatory response with hypersecretion of mucus, which cleared in one month.

The data of Ishihara et al. was analyzed using benchmark dose methodology. Doses were calculated as mg Ni^{2+} inhaled with the average body weight of 0.289 kg and the relation Inhalation in rats (m^3/day) = 0.105*(bodyweight, kg/0.113)$^{2/3}$. Adequate model fits ($P \geq 0.1$) were obtained for continuous benchmark doses at the one standard deviation point with linear, power or polynomial models. The 95% lower bounds on the benchmark doses for a one standard deviation change in the respective endpoints ($BMDL_{1SD}$) were 5.5 µg (linear, $P = 0.132$), for total cells/µL BALF; 18.6 µg (power, $P= 0.156$), for total protein mg/mL BALF; 50 µg (polynomial, $P = 0.19$), for total elastolytic activity as nmol succinyl trialanine *p*-nitroanilide hydrolyzed/hr/mL BALF; and 13.5 µg (power, $P = 0.34$) for sialic acid µg/mL BALF. For the five hours/day times five days inhaled exposure volume of 0.2 m^3, the $BMDL_{1SD}$ equivalent concentrations for the four endpoints would be 27.5, 93, 250, and 67.5 µg/m^3, respectively. These values appear significantly lower than the BMDL of 165 µg/m^3 for inhibition of antibody production in mice (data of Graham et al., 1978).

Mongan et al. (2008) studied the role of mitogen activated protein kinase kinase kinase 1 (MAK3K1) in nickel-induced acute lung injury in mice. Wild type mice and MAK3K1 deficient mutants were exposed to $NiSO_4 \cdot 6H_2O$ aerosol (MMAD = 0.2 µm) at 150 µg/m^3 continuously and survival times recorded. Inactivation of one functional allele in *Map3k1*$^{+/\Delta KD}$ heterozygous mutants did not alter survival; however, *Map3k1* homozygous mutants died significantly sooner than wild type control mice. Wild type and heterozygous mutants showed 20% survival at 110 hr compared to 20% survival at 80 hr for the homozygous mutants ($N = 6$ mice/group, $P < 0.01$ by *t*-test). During exposure, the mice developed severe dyspnea, with gross lung pathology showing air trapping and extensive hemorrhagic edema indicative of acute lung injury. Other experiments carried out in vitro with mouse embryo fibroblast cells indicate that MAK3K1 protects against lung injury by inhibiting the Ni-induced activation of c-jun N-terminal kinases (JNKs).

Singla et al. (2006) found that acute oral administration of $NiSO_4$ (50 mg/kg-d x 7 days) to rats affected the structural and functional integrity of the intestine. The activities of the brush border enzymes maltase ($P < 0.05$), lactase ($P < 0.05$), alkaline phosphatase ($P < 0.05$) and leucine amino peptidase ($P < 0.05$) were increased in purified brush borders from Ni-treated rats compared to controls. Alternatively, sucrose, trehalase ($P < 0.01$) and glutamyl transpeptidase ($P < 0.05$) were reduced in nickel fed animals compared to controls. Kinetic analysis of alkaline phosphatase and sucrose indicated that quantity of enzymes (Vmax) was altered by nickel exposure rather than their activity (Km). Regional analysis indicated that the changes in enzyme activity were mainly located in the villus tip and mid villus regions, rather than the crypt base. The authors conclude that acute feeding of nickel affects the development of various brush border enzymes along the crypt-villus axis of the rat intestine.

6.1 Acute Toxicity Summary

The acute toxicity of inhaled nickel compounds is affected by their solubility and particle size distribution. Similar toxic effects were seen in both exposed humans and experimental animals, primarily lung lesions and decreased lung function and immunotoxicity. These toxic endpoints, pneumotoxicity and immunotoxicity, appear to form the best basis for deriving acute and 8-hour reference exposure levels.

7. Reproductive or Developmental Toxicity

7.1 Human studies

Chashschin et al. (1994) reported that an increase in spontaneous abortions was observed among 290 women (15.9%) who worked in a nickel hydrometallurgy refining plant in Russia, compared with 336 female construction workers without any occupational nickel exposure as controls (8.5%). The workers were exposed to primarily nickel sulfate at 0.11 to 0.31 mg Ni/m^3. In the same study, the authors also noted a statistically significant increase in structural malformations among offspring born to 356 workers (16.9%) compared to 342 controls (5.8%). They reported relative risks were 2.9 for all kinds of defects, 6.1 for cardiovascular system defects, and 1.9 for musculoskeletal defects. They noted heavy manual activity and heat stress as potential confounders. No confidence intervals or other statistical analyses were provided by the authors.

Benoff et al. (2000) studied the effects of metal ions on human sperm mannose receptor expression, a biomarker of spermatotoxicity. Exposure of human sperm to Ni(II) had a biphasic effect with a low concentration of 4.21 nM Ni(II) stimulating the mannose receptor expression ($P < 0.01$) and higher concentrations of 421 nM and 42 µM Ni(II) decreasing expression ($P < 0.014$).

Danadevi et al. (2003) studied semen quality of Indian welders occupationally exposed to nickel and chromium. Fifty-seven workers from a welding plant in South India and 57 controls were monitored. Blood nickel and chromium concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS). World Health Organization

criteria were employed in analyzing semen samples. The nickel and chromium blood concentrations for 28 exposed workers were 123.3±35.2 and 131.0±52.6 µg/L, respectively. The control levels (N = 27) were much lower at 16.7±5.8 and 17.4±8.9 µg/L, respectively. Sperm concentrations were 14.5±24.0 x 10^6/mL for exposed workers vs. 62.8±43.7 x 10^6/mL in the controls. Rapid linear sperm motility was decreased in the exposed subjects compared to controls and there was a significant positive correlation between the percentage of sperm tail defects and blood nickel in exposed workers (R = 0.485, P = 0.036). These investigators also report a negative correlation of sperm concentration with blood chromium in exposed workers (R = -0.424, P = 0.025).

Vaktskjold et al. (2006) investigated the incidence of genital malformations in newborns of women nickel-refinery workers. In this register-based cohort study, data about pregnancy outcome and occupation were obtained from the Kola Birth Registry. The reference population comprised delivering women from Moncegorsk. The association of the outcome with assigned exposure ratings was analyzed with a logistic regression model, adjusted for parity, maternal malformation, exposure to solvents and infection in early pregnancy. There was no association between nickel exposure and genital malformations in this study. The odds ratio (OR) for nickel-exposed women delivering a newborn with a genital malformation was 0.81(95% C.I. 0.52-1.26) and that for undescended testicle was 0.76(95% C.I. 0.40-1.47). The study is limited by few cases in the higher exposure groups.

Vaktskjold et al. (2008a) studied the incidence of musculoskeletal defects in the offspring of women occupationally exposed to nickel in early pregnancy. Nickel exposure was characterized by using as a guideline the water-soluble Ni subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel- and copper-refinery workers and/or measured urinary-Ni concentrations. In total, the study population consisted of 22,965 births. Three hundred and four infants (13.3/1000 births; 95% C.I. 11.9-14.7) were diagnosed with isolated musculoskeletal defects(s) at birth. The adjusted odds ratio for the association between maternal exposure to nickel and the observed defects was 0.96 (95% C.I. 0.76-1.21). The authors concluded that despite the high incidence of defects there was no apparent association with maternal nickel exposure.

Vaktskjold et al. (2008b) studied the incidence of spontaneous abortion among nickel-exposed female refinery workers. A case-control study involved women employed in nickel-exposed work areas in early pregnancy. Each pregnancy record was assigned a categorical nickel exposure rating according to occupation at pregnancy onset. The guidelines were the water-soluble Ni subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel- and copper-refinery workers and/or measured urinary-Ni concentrations. The cut-off between low and high exposure levels was 70 µg Ni/L urine corresponding to about 160 µg Ni/m^3 of the water soluble sub-fraction. The unadjusted OR for the association between maternal Ni exposure and spontaneous abortion was 1.38 (95% C.I. 1.04-1.84), and the adjusted OR was 1.14 (95% C.I. 0.95-1.37). Adjustments included previous induced abortion, previous delivery, solvent or paint exposure, heavy lifting, and maternal age >34 years. Addition of maternal smoking did not significantly change the OR, 1.15(0.96-1.39). The authors concluded that no

statistical association was established; however they note that the findings do not exclude the possibility of a weak excess risk.

7.2 Animal studies

7.2.1 Reproductive Toxicity

Schroeder and Mitchener (1971) conducted a three-generation reproduction study in Long-Evans rats administered drinking water containing five mg Ni/L (0.43 mg Ni/kg-d, U.S.EPA, 1988). Five pairs of rats were randomly selected at the time of weaning, placed in separate cages and given nickel in drinking water *ad libitum*. The rats were allowed to breed for up to nine months of age or longer. At weaning, pairs were randomly selected from the first, second and third litter (F_1) and allowed to breed and to produce the F_2 generation. Pairs were likewise selected at random from the F_2 litters to breed the F_3 generation. They observed that all nickel-exposed animals in the three generations gave birth to litters that exhibited significantly increased perinatal mortality ($P < 0.0001$), and there was a significantly increased number of "runts" in the first ($P < 0.025$) and third ($P < 0.0001$) generations. The study suffers from small sample size, and the fact, that matings were not randomized and that males were not rotated.

Ambrose et al. (1976) studied the effects of dietary administration of nickel sulfate hexahydrate in a three-generation study in rats. Male and female rats in the parent generation were exposed to 0, 250, 500, or 1000 ppm nickel, starting at 28 days of age. Mating was initiated after 11 weeks of nickel exposure. Rats in the first, second and third generations were also given the same diet as the parent generation. At each mating, 20 females from each dose level were transferred to individual breeding cages and mated with a male from the same dietary nickel level. The authors did not observe any adverse effect on fertility, pregnancy maintenance, or postnatal survival of offspring in the three generations. They did report a dose-dependent decrease in the number of siblings weaned per litter averaging 8.1, 7.2, 6.8, and 6.4, respectively. Weanling body weight was clearly affected at the top dose level averaging 73% of the controls. The study suffers from small sample size and the use of pups rather than litters as the unit of comparison.

In a two-generation study (RTI, 1988), nickel chloride was administered in drinking water to male and female CD rats (30/sex/dose) at dose levels of 0, 50, 250, or 500 ppm (mg Ni^{2+}/L) for 90 days before breeding. A significant decrease in the P_0 maternal body weight was observed at the highest dose level. A significant decrease in live pups/litter and average pup body weight versus controls was also seen at the 500 ppm level in the F_{1a} generation. Similar effects were seen in the F_{1b} litters of P_0 dams exposed to the 500 ppm dose level. Increased pup mortality and decreased live litter size were also observed in the 50 and 250 ppm dose groups in the F_{1b} litters. These latter findings are questionable due to increased temperature and humidity experienced by the F1b litters, which could have influenced the observed effects (Edwards, 1986). F1b males and females were randomly mated on postnatal day (PND) 70 and their offspring were evaluated through PND 21. The 500 ppm dose level caused a significant body weight depression of both mothers and pups, and increased neonatal mortality. The 250 ppm

dose level produced transient depression of maternal weight gain and water intake during gestation of the F2b litters. A significant increase in short ribs was observed in the 50 ppm dose group, but since this was not seen in the higher doses, it is not considered to be biologically significant.

Kakela et al. (1999) evaluated the effect of $NiCl_2$ administered in drinking water on reproduction in Wistar rats. Four groups of six female rats were exposed to 10-100 ppm Ni^{2+} for up to100 days prior to conception and through gestation and lactation. Two groups of male rats were exposed to 30 ppm Ni^{2+} for 28 and 42 days prior to conception and one group of males and females were exposed to 30 ppm Ni^{2+} for 28 days prior to conception. Exposure was continued for the females through lactation. The males were sacrificed at conception. When males were exposed to Ni^{2+} both the number of pregnancies and the number of pups born were reduced. The control value for gestation index (number of live pups per dam) was 10.2 ± 1.5 SE versus 2.7 ± 1.4 ($P < 0.01$) for 28 day exposures and 7.8 ± 2.0 for 42 day exposures. The litter sizes were 9.2 ± 1.5, 1.3 ± 0.9 ($P < 0.01$), and 6.2 ± 2.0, respectively. Females exposed to100 ppm Ni^{2+} 14 days prior to conception also gave reduced litters: 4.0 ± 1.0 ($P < 0.05$). Histological examination of testes in nickel-exposed rats revealed shrinkage of the seminiferous tubules and decreased number of basal spermatogonia. When both parents were exposed to nickel, pup mortality during lactation was high.

NiPERA (2000a) sponsored a one-generation reproduction study in Sprague-Dawley rats with nickel sulfate hexahydrate. Eight animals per sex/dose group were administered 0, 10, 20, 30, 50 or 75 mg/kg-d by daily aqueous gavage to the F0 parental animals and selected F1 offspring. Dosing of the F0 animals began two weeks prior to mating and dosing of F1 offspring began on PND 22. All doses were given at constant volume of 10 mL/kg. Both F0 and F1 animals were examined for indications of toxicity. Experimental endpoints for F0 animals included clinical observations, body weights, food and water consumption, mating, parturition, lactation and offspring growth and viability. Experimental endpoints for selected F1 animals included survival, clinical observations and body weight during the F1 dosing phase. All F0 and F1 animals were subjected to gross necropsy examination at time of death or terminal sacrifice. For the F0 animals post-implantation loss (implantation scar count minus the number of live pups on Day 0) was significantly increased at the 30, 50, and 75 mg/kg-d dose levels and increased at the 10 and 20 mg/kg-d levels (mean loss values: 0.4 control; 2.6; 1.5; 2.3($P<0.05$); 2.7 ($P<0.01$); 4.8($P<0.01$)). For F1, pup viability was significantly decreased at all dose levels except 50 mg/kg-d compared to the control (dead/live: 1/128 control; 12/100; 10/106; 10/92; 4/89; 23/80 all $P < 0.01$ except 50 mg/kg-d). For this study a LOAEL of 10 mg/kg-d equivalent to 2.1 mg Ni/kg-d was identified.

NiPERA (2000b) sponsored a two-generation reproduction study in Sprague-Dawley rats with nickel sulfate hexahydrate. Twenty-eight animals per sex per group were administered 0, 0.22, 0.56, 1.12, or 2.23 mg Ni/kg-d by aqueous gavage. The animals were exposed from ten weeks prior to mating for F0, through gestation, and until PND 21 (13 weeks to delivery of F1 offspring). Exposure for F1 was from *in utero*, during lactation, through development from PND 22 to about PND 92 (a minimum of 70 days of

treatment). In contrast to the one-generation study the F0 animals showed no statistically significant effects of nickel administration on implantation and post-implantation losses. Statistically significant differences in F0 organ weights consisted of decreased absolute and relative liver weights in the high dose males, decreased absolute brain weight in the mid dose females, and increased relative liver weight in 0.56, 1.12 and 2.23 mg/kg-d group females. The investigators did not consider these organ weight effects to be toxicologically meaningful. The percent of dead pups/total in the respective dose groups were: 2.2 (control); 3.7; 2.2; 2.1; 4.2 ($P = 0.105$ vs. control by one-sided Fisher exact test). For this study a NOAEL of 2.23 mg Ni/kg-d was identified by the authors.

Administration of 25 µmol Ni/kg-d for 5 days only marginally affected mating efficiency of males (75% vs. 80-90% in controls). No significant difference was seen in the total number of implantations among pregnancies resulting from nickel-treated males. Total implantations/litter from nickel-treated males ranged from 10.9 to 11.4. However there was a marked decrease in the number of live implantations among the nickel animals during weeks 1 to 3. The mean incidence of dead implantations during these three weeks was 1.9, 3.2, and 2.2, respectively (all $P < 0.05$ vs. control). These values compare with those for a single 100 mg/kg dose of cyclophosphamide, a dominant lethal mutagen, of 5.3, 6.33, and 3.6, respectively (all $P < 0.001$ vs. control). The percentage of dead implantations/litter expressed as a percentage of total implants for weeks 1, 2, and 3 were: control, 8.69, 8.03, 10.9; nickel, 16.5 ($P < 0.05$), 28.00 ($P < 0.001$), 19.64 ($P < 0.001$); cyclophosphamide, 60.27, 55.86, 35.00 (all $P < 0.002$). The results clearly suggest a specific Ni-induction of dominant lethal-type mutations.

7.2.2 Developmental Toxicity

There are several reports of teratogenicity and other reproductive effects in laboratory animals exposed to nickel (Ambrose et al., 1976; Schroeder and Mitchener, 1971; RTI, 1987; Smith et al., 1993). Mice exposed during pregnancy to $NiCl_2$ by intraperitoneal injection bore offspring with numerous fetal malformations and skeletal anomalies. In addition there were increased fetal resorption rates and decreased fetal weights (Lu *et al*., 1979). Woollam (1972) showed that nickel acetate, when injected intraperitoneally into pregnant hamsters, caused significant fetal mortality at 25 mg/kg.

Intravenous exposure of pregnant rats to 11 mg Ni/kg caused increased fetal mortality and a 16% incidence of fetal malformations including anopthalmia, cystic lungs, and hydronephrosis (Sunderman *et al*., 1983). Temporary hyperglycemia was seen in pregnant rats exposed intraperitoneally to $NiCl_2$ at four mg/kg (Mas *et al*., 1985). The authors proposed that this hyperglycemia was a mechanism for teratogenicity.

Sunderman et al. (1978) administered nickel chloride (16 mg Ni/kg) to Fischer rats by intramuscular (i.m.) injection on day eight of gestation. The body weights of fetuses on day 20 of gestation and of weanlings four to eight weeks after birth were reduced. No congenital anomalies were found in fetuses from nickel-treated dams, or in rats that received 10 i.m. injections of 2 mg Ni/kg as nickel chloride twice daily from day 6 to day 10 of gestation.

Diwan et al. (1992) showed that intraperitoneal (i.p.) injection of nickel acetate to pregnant F344/NCr rats caused early mortality in the offspring. They administered four i.p. injections of nickel acetate (2.6 mg Ni/kg) on days 12, 14, 16, and 18 of gestation and reported that all offspring died within 72 hr after birth.

Smith *et al.* (1993) administered nickel chloride in drinking water at 0, 10, 50, or 250 ppm (0, 1.3, 6.8, or 31.6 mg/kg-d) to 34 female Long-Evans rats per group for 11 weeks before mating and subsequently during two sequential gestations (G1, G2) and lactation (L1, L2) periods. Pups were observed until weaning and breeder males were unexposed. Dams were rested for two weeks after weaning of the first litters before initiating the second breeding. During this time exposure to nickel was continuous. The animals were 6-7 months old when they produced their second litters. Throughout the study, there were no overt clinical signs of toxicity in any of the dose groups. Reproductive performance was unaltered by nickel exposure although maternal weight gain was reduced during G1 in the mid and high dose groups. The most significant finding was the increased frequency of perinatal death (Table 4). The authors reported that the proportion of dead pups per litter was significantly increased at the highest dose level in G1 ($P \leq 0.01$) and at the low ($P \leq 0.03$) and high ($P \leq 0.01$) dose levels in G2. The mid dose level in G2 was also increased ($P = 0.076$). Overall there was a dose related increase in perinatal mortality in both segments of the study. The authors concluded that 10 ppm $NiCl_2$ (1.3 mg Ni/kg-d) represented the LOAEL in the study.

Slotkin and Seidler (2008) evaluated the effects on Ni^{2+} in a neurodevelopmental cell model. Neurodifferentiating rat PC12 pheochromocytoma cells were treated with 30µM $NiCl_2$. The cell cultures were examined 24 and 72 hr after the start of exposure with five to eight independent cultures at each time point. Unlike organophosphorus (OP) agents studied with this system, nickel reduced expression of tryptophan hydroxylase (*tph*) and enhanced vesicular monoamine transporter (*slc6a4*). Nickel exposure reduced the net expression of 5HT receptor genes more effectively than did diazinon or dieldrin. Significant decrements were seen for *htr1d*, *htr2a* and *htr3b*. The authors conclude that the results provide "evidence connecting the direct, initial mechanisms of toxicant action on specific neurotransmitter pathways with their long-term effects on synaptic function and behavior."

Table 4. Reproductive Outcomes of Breeding Female Rats Exposed to Nickel Chloride in Drinking Water (Smith *et al*., 1993).

Concentration of nickel in water ppm Ni (No. females)	Sperm positive females	No. viable litters	Average no. of pups per litter (live and dead)	No. of litters with dead pups at birth	Total dead pups on post natal day 1(% dead pups per litter)
G1, L1					
0 (34)	29	25	12.9	5	5 (1.7)
10 (34)	30	25	12.2	5	9 (3.1)
50 (34)	30	24	11.7	0	0 (0)
250 (34)	32	27	13.2	11	35*** (13.2)**
G2, L2					
0 (29)	28	23	10.6	2	2 (1.0)
10 (29)	28	22	12.5	7	11** (4.3)**
50 (30)	29	24	13.3	6	16* (4.6)
250 (31)	31	25	11.3	10	22*** (8.8)***

Note: Significant levels, pairwise comparison to control: * $0.05 < P \leq 0.10$; ** $0.01 < P \leq 0.03$; *** $0.001 < P \leq 0.01$.

7.2.3 Testicular Effects

Male rat reproductive toxicity (damage to epididymal tubules and abnormal spermatozoa) was observed following a single subcutaneous dose of 5 mg Ni/kg as Ni_3S_2 (Hoey, 1966). Benson et al. (1987) showed that mice and rats exposed to 5 or 10 mg Ni_3S_2/m^3 displayed degeneration of testicular germinal epithelium after 12 days exposure (6 hours/day, 5 days/week).

Pandey et al. (1999) administered $NiSO_4$ orally to adult male mice at 0, 5 or 10 mg/kg bw for 5 days/week for 35 days. Significant dose-dependent decreases were observed in absolute and organ-to-body weight ratios of testes, epididymides, seminal vesicles, and prostate gland. Also observed were decreases in sperm motility and count. Significant alterations of marker testicular enzymes were seen: γ-glutamyl transpeptidase, 28.76, 35.23, and 38.44*; sorbitol dehydrogenase, 7.88, 6.00, and 4.04*; and lactate dehydrogenase, 194.2, 236.7, 243.9*, respectively (* $P<0.05$, N=10, all activities nmol/min/mg protein).

Pandey and Srivastava (2000) reported spermatotoxic effects of nickel in mice. Young male mice (25 ± 5 g), six/dose group were administered 0, 5, 10, or 20 mg/kg bw of $NiSO_4$ or $NiCl_2$ orally by gavage in 0.2 mL distilled water five days/week for 35 days. The animals were sacrificed on day 36 and the testes, epididymides, seminal vesicles and

prostate glands were removed and weighed. No overt toxicity was observed. The absolute and relative weights of testes, epididymides, seminal vesicles and prostate gland were significantly decreased at the top dose of 20 mg/kg bw. Dose-dependent reductions in sperm motility were observed at 10 and 20 mg/kg bw with nickel sulfate and nickel chloride ($P < 0.05$). Dose-dependent decreases in sperm count were also seen with both nickel compounds but were statistically significant only at the top dose with $NiSO_4$. There was a significant increase in abnormal sperm including abnormalities of the head, neck and tail region. Curved neck and curved, bent, round, loop and folded tail were seen at both higher doses with $NiSO_4$ and $NiCl_2$. A continuous benchmark dose analysis of the sperm motility and sperm count data gave only one adequate fit, namely decrease in motility with $NiSO_4$ treatment ($BMDL_{1SD}$ = 2.91 mg/kg bw, linear model, $P = 0.22$). A similar analysis of sperm abnormality data gave adequate fits for both compounds: $NiSO_4$, $BMDL_{1SD}$ = 0.46 mg/kg, polynomial model, $P = 0.97$; and $NiCl_2$, $BMDL_{1SD}$ = 0.34 mg/kg, polynomial model, $P = 0.12$.

Xie et al. (1995) evaluated the effects of chelating agents on testicular toxicity in mice caused by acute nickel exposure. Male ICR mice were injected intraperitoneally with $NiCl_2 \cdot 6H_2O$ at doses of 0, 0.5, 1.0, 3.0, or 5.0 mg Ni/kg bw and sacrificed 24 hr after injection. Nickel administration resulted in dose-dependent increases in testicular lipid peroxidation (LPO), and Ni, calcium (Ca) and iron (Fe) concentrations (all $P < 0.05$, N=5). Lesser increases in testicular copper (Cu) and zinc (Zn)were also seen. Treatment with 5.0 mg Ni/kg and seven days observation showed increasing LPO with a peak at two days after Ni administration followed by a gradual decrease. Testicular weight decreased from about 0.65% of body weight to 0.4% over the same period ($P < 0.05$, $N = 5$). Among five chelating agents tested *meso*-2, 3-dimercaptosuccinic acid (DMSA) and *N*-benzyl-D-glucaminedithiocarbamate (BGD) were the most effective in removing nickel from the testes, protecting against LPO and Ni-induced sterility.

Das and Dasgupta (1997) treated male Wistar rats with 20 mg $NiSO_4$/kg bw by intraperitoneal injection on alternate days for 20 days. Significant decreases were observed in testicular weight, lactate dehydrogenase, and protein concentration and increases in testicular glycogen and cholesterol (all $P < 0.05$, $N = 6$). The differences from control animals were generally enhanced in parallel groups fed a protein-restricted diet with or without nickel sulfate administration.

Forgacs et al. (1998) evaluated the effects of Ni(II) on testosterone production of mouse Leydig cells in vitro following repeated in vivo or in vitro exposures. CFLP mice were injected s.c. (four treatments every three days) with 0, 10, 20, or 40 mg $NiSO_4 \cdot 7H_2O$/kg bw. Human chorionic gonadotropin (hCG)-stimulated testosterone response was reduced by Ni-treatment in 48 hr cultures of testicular interstitial cells from treated animals in a dose-dependent manner (100 (control), 88%, 80%*, and 59%*, respectively (* $P < 0.05$, $N = 4$)). Direct nickel effects were assessed in 48 hr cultures of hCG-stimulated testicular interstitial cells exposed to 0, 62.5, 125, 250, 500, or 1000 µM $NiSO_4$. Testosterone production relative to hCG control was 100% (control), 105%, 78%*, 56%*, 32%*, and 18%* respectively (* $P < 0.05$, $N = 7$). Cytotoxicity was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay following

exposure and cell viability remained above 80% at all doses. The data indicate that the effect of nickel on the Leydig cell testosterone production is time and concentration dependent, and is not due to cytotoxicity.

Das and Dasgupta (2000) treated male Wistar rats with 20 mg $NiSO_4$/kg bw by intraperitoneal injection on alternate days for 20 days. Significant decreases in cauda epididymal sperm count and sperm motility were observed following treatment ($P < 0.05$). In addition decreases were seen in testicular DNA, RNA, and total protein concentrations ($P < 0.05$). The authors conclude that $NiSO_4$ is a likely gonadotoxicant that adversely affects the expression of genetic information via reduced nucleic acids and protein. In a subsequent study using a similar protocol in male rats Das and Dasgupta (2002) found that nickel treatment significantly reduced the activities of two testicular steroidogenic enzymes, 3β- and 17β-hydroxy steroid dehydrogenases (HSD), and plasma testosterone concentration. 3β-HSD was reduced from 8.97 ± 0.18 in control normal protein diet rats to 6.57 ± 0.23 units/mg ($P < 0.05$) in normal diet plus $NiSO_4$. For 17β-HSD the reduction was from 6.50 ± 0.29 to 5.10 ± 0.21 units/mg protein ($P < 0.05$), respectively. Plasma testosterone was reduced from 3.27 ± 0.06 to 2.43 ± 0.10 ng/mL ($P < 0.05$), respectively. Increases in testicular cholesterol and ascorbic acid were observed in the same groups of rats. Some reversibility of the effects was seen when treated animals were fed a normal diet during a withdrawal period.

Doreswamy et al. (2004) treated adult male CFT-Swiss mice with 0, 12.5, 25, or 50 µmol $NiCl_2$/kg bw/d by single i.p. injection for three or five treatments. The mice were sacrificed 24 hr after the final dose and evaluated for biochemical endpoints, DNA damage and fragmentation and at 1, 2, 3, and 5 weeks from the beginning of treatment for sperm head abnormalities. No clinical signs of toxicity were observed at any administered dose. Dose-dependent increases in lipid peroxidation were seen with whole testicular homogenates (10-25%), mitochondrial fractions (20-45%), microsomal fractions 25-60%), and epididymal sperm (8-25%). Antioxidant enzymes were similarly increased: glutathione peroxidase (8-26%); glutathione *S*-transferase (15-26%); and catalase (10-25%). Nickel treatment also resulted in a dose-dependent decrease in double stranded DNA (ds-DNA) in the testis and in epididymal spermatozoa. For testis the proportion of ds-DNA was 83% (control), 80%, 65% ($P < 0.05$), and 62% ($P < 0.05$), respectively. For epididymal sperm the values were 90%, 85%, 82% ($P < 0.01$), and 80% ($P < 0.01$), respectively. Agarose gel electrophoresis of genomic DNA, visualized by ethidium bromide fluorescence, showed evident DNA damage at 6.25, 12.5, 25.0 and 50 µmol Ni/kg-d for three days. Caudal sperm counts did not differ from the control. However, nickel treatment induced a significant dose-dependent increase in the percentage of abnormal sperms, mainly amorphous heads, balloon heads, and hammerheads.

7.3 Reproductive and Developmental Toxicity Summary

Human studies of workers exposed to nickel compounds by the inhalation route suggest increased incidence of spontaneous abortions in females and spermatotoxicity in males. In experimental animals no inhalation studies were identified but oral exposures resulted in spermatotoxicity in mice and rats involving both induction of mutation and endocrine disruption, and reduced reproduction in rats (both sexes exposed separately and together). Nickel-exposed mice and rats also exhibited significantly increased perinatal mortality.

8. Chronic Toxicity Human Studies

8.1 Pneumotoxicity

A number of studies indicate that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey et al. (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell et al., 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by FEV_1 with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal values. This worker reported dyspnea, non-productive cough, chest-tightness, and wheezing as symptoms during the work period.

A group of seven metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla et al., 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the seven asthmatics exhibited significantly decreased FEV_1 (> 15%) when exposed to 0.3 mg/m^3 nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV_1.

Fernandez-Nieto et al. (2006) reported results obtained from four patients with work-related asthma due to exposure to metallic salts. Two subjects came from factories where potassium dichromate and nickel sulfate were used for electroplating, another worked in a cement factory (potassium dichromate), and one was a welder exposed to metal fumes, including nickel and chromium. All the patients had bronchial hyperresponsiveness (BH) to methacholine, which increased 24 hr after challenge with metal salts. Airway hyperresponsiveness to methacholine was assessed as the provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}). The methacholine inhalation test was performed the day before the antigen challenge and again 24 hr after challenge. A two-fold or greater reduction of the PC_{20} compared to baseline value was considered a significant change. Nickel sulfate challenge of subject 1 (electro-plating) elicited a BH

response at a methacholine concentration of 10 mg/mL and in subject 2 (cement) of 0.1 mg/mL. Twenty-four hours after nickel challenge, the PC_{20} for subject 1 was 0.15 mg/mL.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis although an increase in irregular lung opacities was observed by Muir et al. (1993) with exposures ≥ five years in 149 nickel sinter plant workers. Pang et al. (1996) observed slight but not statistically significant increased relative risk of mortality due to non-malignant diseases of the respiratory system in nickel platers exposed to $NiCl_2$ and $NiSO_4$. The relative risk with adjustment for age, period of follow up, and year starting nickel work was 1.59 (95% CI, 0.58 to 4.36). The study suffers from low numbers (248 subjects total) and relatively brief soluble nickel exposures (mean = 2.1 yr, median 0.86 yr). An occupational epidemiology report by Broder et al. (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter.

Moulin et al. (2000) conducted a mortality study of 4898 stainless steel workers exposed to metallic alloys including nickel. Among the non-malignant endpoints included, no significant increases in standardized mortality ratios (SMRs) for chronic bronchitis, pneumoconiosis or other respiratory system effects were seen. Huvinen et al. (2002) studied 284 workers in a ferrochromium and stainless steel plant. Long-term workers (average 23 years) exposed to low levels of dusts and fumes containing molybdenum (0.3 $\mu g/m^3$), nickel (1.8 $\mu g/m^3$) and chromium (4.7 $\mu g/m^3$) did not show evidence of respiratory disease detectable by lung function tests or chest radiography. Similarly, Egedahl et al. (2001) studied mortality experience among employees at a hydrometallurgical nickel refinery and fertilizer complex in Alberta, Canada. A total of 1649 males who worked continuously for at least 12 months during the years 1954 to 1978 were followed for an additional 17 years. Exposure with this refining process involves nickel metal rather than soluble nickel or sulfides. The observed deaths due to respiratory disease were less than expected (SMR = 36, C.I. 13 to 79).

Berge and Skyberg (2003) reported evidence of increased radiographic lung abnormalities with increased exposure to soluble or sulfidic nickel, albeit with a relatively small number of cases (47/1046) and relatively mild effects. Pulmonary fibrosis was defined as a median reading of International Labor Organization (ILO) score ≥ 1/0. For soluble nickel exposure the crude odds ratio for pulmonary fibrosis was 4.34 (95% CI, 1.75 to 10.77). The risk adjusted for age, smoking, asbestos, and sulfidic nickel was 2.24 (95% CI, 0.82 to 6.16) with a dose-response. The corresponding values for sulfidic nickel were crude 5.06 (95% CI, 1.70 to 15.09) and adjusted, as above except for substituting soluble nickel for sulfidic nickel, 2.04 (95% CI, 0.54 to 7.70). The prevalence values for pulmonary fibrosis and both soluble and sulfidic cumulative nickel exposure (their Tables 5 and 6) were acceptably fit by the multistage model. For soluble nickel a $BMDL_{01}$ (1 % excess risk) of 0.35 (mg Ni/m^3)-yr was obtained ($\chi^2 = 2.21$, P = 0.33). For sulfidic nickel the $BMDL_{01}$ was 0.19 (mg Ni/m^3-yr, $\chi^2 = 3.91$, P = 0.14). Dose responses on the adjusted data sets were not fit as well by the model as were the crude data. For example the soluble nickel gave a $BMDL_{01}$ of 0.69 ($\chi^2 = 3.11$, P = 0.08)

when adjusted for smoking, age, asbestos and sulfidic Ni (g-adjustment) and a $BMDL_{01}$ of 0.56 (mg/m^3)-yr ($\chi^2 = 1.72$, P = 0.42) when adjusted for age, smoking and asbestos only (f-adjustment). For sulfidic nickel no BMD or BMDL could be calculated from the g-adjusted data sets and with f-adjustment the $BMDL_{01}$ was 0.34 (mg Ni/m^3)-yr (χ^2 = 4.16, P = 0.125). As the authors note, the data are not strong but there is a measureable dose response for cumulative nickel exposure and pulmonary fibrosis. The mean and median exposure periods were 21.8 and 21.9 years, respectively.

Sivulka et al. (2007) reviewed the literature on nickel exposure and non-malignant respiratory disease and suggested that the failure to observe frank lung toxicity in exposed nickel workers may be related to the particle size to which the workers were exposed. The authors point out that in rat studies showing lung lesions, exposures have been to respirable-sized particles (< 4 µm diameter) whereas occupational exposures constitute largely non-respirable larger diameter particles.

8.2 Immunotoxicity

Dermal exposure to nickel and nickel alloys has long been known to cause dermatitis in both nickel workers and the general population. A number of studies indicated that oral exposure of nickel could aggravate nickel dermatitis in people who are sensitive to nickel. Christensen and Möller (1975) found that oral administration of nickel (approximately 5 mg) in diet worsen hand eczema in nickel-allergic patients. In a clinical trial, Kaaber et al. (1978) reduced the nickel dose to 2.5 mg and observed flaring of hand dermatitis in 13 of the 28 patients with chronic nickel dermatitis. A similar finding was reported by Veien et al. (1983); they observed that 26 patients had flare-ups following oral challenge with nickel compounds (2.5 mg nickel in a capsule). The conditions of some of the patients improved when they were placed on a low-metal allergen diet for four to six weeks (Kaaber et al., 1978; Veien et al., 1983).

Cronin et al. (1980) gave groups of five fasting female patients that had hand eczema a gelatin lactose capsule containing nickel, together with 100 ml of water. Three doses were used, 2.5 mg, 1.25 mg, and 0.6 mg nickel as nickel sulfate. After administration of nickel, the fast was continued for a further hour, at which time the patient was given a cup of coffee; thereafter, normal meals were taken. Assuming a female body weight of 62 kg (OEHHA, 2000b, p10-4) and the lowest dose that aggravated nickel dermatitis of 0.6 mg; we estimate a LOAEL of 9.7 µg Ni/kg bw.

Nielsen et al. (1999) studied the aggravation of nickel dermatitis in people by giving them an oral dose of soluble nickel. Twenty nickel-sensitized women and 20 age-matched controls, both groups having vesicular hand eczema of the pompholyx type, were given a single dose of nickel in drinking water (3 µg/mL or 12 µg Ni/kg bw). All patients fasted overnight and fasting was maintained for another 4 hours after the nickel administration. Nielsen et al. (1999) reported that nine of 20 nickel-allergic eczema patients experienced aggravation of hand eczema after nickel administration, and three also developed a maculopapular exanthema. No exacerbation was seen in the control group. From the results of this study, we identified a LOAEL of 12 µg Ni/kg bw for the nickel-sensitized women.

A number of human studies have shown that oral administration of low levels of soluble nickel over a long period of time may reduce nickel contact dermatitis. Sjovall et al. (1987) orally administered 0, 5 or 0.5 mg nickel per day to a group of patients allergic to nickel. After six weeks, they found evidence of reduced sensitization in patients exposed to 5 mg/day but not to 0.5 mg/day. Santucci et al. (1988) gave a single oral dose of 2.2 mg Ni to 25 nickel-sensitized women and found that 22 reacted to the treatment. After a 15-day rest period, the subjects were given gradually increasing doses under the following schedule: 0.67 mg Ni/day for one month, 1.34 mg Ni/day for the second month, and 2.2 mg Ni/day for the third month. In the last phase of the testing, 3/17 of the subjects had flare-ups even at the lowest dose. The other 14 subjects, however, did not respond to the highest dose, even though they had responded to that dose in the initial testing.

Zeromski et al. (1995) measured the effects of Ni_3S_2 or $NiSO_4$ on human lymphocytes in vitro. Blood was obtained from a blood bank and peripheral mononuclear cells (PBMCs) from normal donors were cultured for 24 hr at 0, 0.01, 0.02, or 0.04 mM Ni. Following culture, the immuno-phenotype of the cells was determined by indirect immunofluorescence, using monoclonal antibodies to major differentiation antigens of PBMCs, and their natural killer (NK) activity toward K562 target cells. Ni_3S_2 had a marked inhibitory effect on the PBMCs consisting of a decreased number of CD4-positive cells at 0.02 and 0.04 mM Ni and a fall of NK (CD56-positive) cell number at all concentrations tested. NiSO4 induced a significant 30 percent decrease in the CD4 phenotype of T cells at 0.04 mM ($P < 0.05$ vs. control). The inhibitory effects noted by both nickel compounds could be prevented by co-treatment with magnesium acetate. Ni or Mg salts did not affect CD3, CD8, CD20, or CD11a cell populations.

Boscolo et al. (1999) evaluated systemic effects of ingested nickel on the immune system of nickel-sensitized women. Twenty-eight women were administered 10 mg of $NiSO_4$. Group A consisted of 19 non-atopic Ni-sensitized or nine non-allergic women. After Ni ingestion non-allergic and 12 Ni-sensitized women were asymptomatic (non-responders, group B) while seven Ni-sensitized women showed a flare up of urticaria and/or eczema (responders, group C). Before Ni treatment, groups B and C showed higher values of blood CD19+ (280 for both groups, vs. 150 pg/mL for Group A, $P < 0.05$) and CD5--CD19+ (235 for B,183 for C, vs. 113 pg/mL for A, $P < 0.05$). Group C also showed higher serum interleukin (IL) 2 (538 vs. 483) and lower serum IL-5 (296 vs. 445, $P < 0.05$) than Group A. Four hours after Ni ingestion, group C showed a significant increase in serum IL-5 (+53.7%, $P <0.05$). Twenty-four hours after treatment, group A showed a significant reduction in blood CD4+-CD45RO- "virgin" cells and an increase of CD8+ lymphocytes, while group C showed a marked decrease in total blood lymphocytes and CD3+(-41.5%), CD4+-CD45RO-(-46.5%), CD4+-CD45RO+(-35.6%), CD8+(-34.6%), CD19+(-28.8%), and CD-CD19+(-20.8%) cell subsets (all $P <0.05$ by Kruskall-Wallis test and/or Wilcoxon matched-pairs signed-rank test). Overall the results suggest that Ni ingestion induces a change in immune response from a TH-1 like pattern to a Th-0 like pattern in responder patients with systemic symptoms, as indicated by elevated serum IL-2 and IL-5 during the test.

Lisby et al. (1999a) observed nickel-induced activation of T cells in individuals with negative patch test to nickel sulfate. Eighteen subjects (8 males and 10 females, aged 27-54 years) were included in the study. Maximum T cell proliferation was seen after seven days of in vitro stimulation of isolated peripheral blood mononuclear cells (PBMC) with $NiSO_4$. Nickel sulfate concentrations above 1.0 mM were toxic to the cells by trypan blue exclusion. At concentrations between 0.1 and 100 µM a dose-dependent stimulation of PBMC was seen in 16 of the 18 subjects. Maximum stimulation occurred between 1 and 100 µM $NiSO_4$ with the mean maximum stimulation index (SI) of 7.1, range 1.4-21.8, ($P < 0.0005$). Similar results were obtained with $NiCl_2$ ($N = 3$, mean SI = 13, range 8.0-20.2). The functional capacity of Ni-inducible T cells was assessed by cytokine release from PBMC from Ni-allergic and Ni-nonallergic individuals. T cells from both allergic and nonallergic subjects released interferon-γ (IFN-γ) but no significant difference was observed between the two groups in the concentrations of IFN-γ released after 72 hr stimulation with $NiSO_4$. Umbilical cord mononuclear cells (UCMC) were used as a model for unexposed individuals. When incubated with 10^{-10} to 10^{-4} M $NiSO_4$ these cells showed no cell proliferation compared to controls. The authors note that: "even if the observed T cell reactivity towards Ni by itself does not result in the development of clinical disease, such a T cell reactivity may add to the reactivity of other T cells with other allergen specificity resulting in the development of overt clinical disease."

In a follow-up study, Lisby et al. (1999b) found that the proliferative response in Ni-nonallergic individuals was mainly confined to T cells within the CD4+ subset. Also in contrast to the conventional recall antigen tetanus toxoid, $NiSO_4$ stimulated both naïve and memory CD4+ T cells. Preincubation of monocytes/macrophages but not T cells with $NiSO_4$ resulted in subsequent T cell proliferation. The results suggest that T cells in Ni-nonallergic individuals are capable of recognizing nickel or nickel-modified peptides.

Buchvald and Lundeberg (2004) investigated the in vitro responses of peripheral blood mononuclear cells (PBMCs) to nickel stimulation in groups of atopic and nonatopic patients with nickel allergic contact dermatitis (ACD). ACD is dependent on cell-mediated immune responses mediated by type-1 T lymphocytes whereas atopic dermatitis (AD) occurs via sustained activation of type-2 subsets of T cells. Ten subjects each with nonatopic nickel ACD, nickel ACD + concomitant AD, AD but no contact allergy, and healthy controls provided PBMCs that were stimulated with $NiSO_4$, phytohemagglutinin (PHA), or tetanus toxoid (TT). Ni-induced lymphocyte DNA synthesis in PBMC cultures was measured with [^{3}H] thymidine incorporation and expressed as a stimulation index (SI). The SI for controls averaged about one, for AD about two, for ACD about 20 and for ACD+AD about two. IL-2 secretion (pg/mL) averaged about 1, 1, 50, and 10, respectively. IL-5 secretion (pg/mL) averaged about 10, 10, 175, and 25, respectively. The results indicated that PBMCs of nickel-allergic subjects with concomitant AD exhibited impaired in vitro proliferative and secretory responses to nickel but not to the mitogen PHA or the recall antigen TT. There was a statistically significant correlation between the amounts of IL-2 and IL-5 secreted by Ni-stimulated lymphocytes of the ACD+AD subjects. The authors speculate that IL-5 may play a role in the development of ACD.

Moed et al. (2004) determined the identity of nickel-responding T cell subsets in five nickel-allergic subjects and four controls. The T cell subsets were isolated from peripheral blood mononuclear cells (PBMCs) and their proliferative capacity, type-1 or type-2, measured by IFN-γ or IL-5 release, and phenotypical marker expression were assessed after nickel treatment with 50 µM $NiSO_4$. The authors found that only CD4+ CLA+ CD45RO+ and not CD8+ T cells proliferated and produced both type-1 and type-2 cytokines in response to nickel. Cells with the marker CLA in combination with CD4+, CD45RO+, or CD69 are increased after nickel stimulation. Analysis of nickel-reactive cells for expression of distinct chemokine receptors showed that proliferative capacity and cytokine production were confined to subsets expressing CXCR3 and CCR4 but not CCR6. A subset of T cells expressing CLA+ and CXCR3, CCR4 and CCR10 increased in response to allergen. The authors conclude that Ni-reactive T cells are characterized as CD4+ CLA+ memory cells, which express chemokine receptors CXCR3, CCR4, and CCR10, but not CCR6. The lack of Ni-induced IFN-γ or IL-5 release from CD8+ T-cell fractions suggests that they play no significant role in nickel allergy.

Jensen et al. (2004) similarly characterized lymphocyte subpopulations and cytokine profiles in PBMCs of Ni-sensitive individuals after nickel exposure. Thirty-three Ni-sensitive individuals were randomly divided into four groups of 7-10 each and orally challenged with 0, 0.3, 1.0, or 4.0 mg nickel given as $NiSO_4 \cdot 6H_2O$. Nineteen healthy controls were randomly divide into two groups and orally challenged with 0 or 4.0 mg Ni. Blood samples were obtained 24 hr after Ni-exposure and PBMCs isolated for analysis. Ni-sensitive individuals had significantly higher fractions of lymphocytes in their peripheral blood than the healthy controls (mean percent): $CD3^+$ $CD45RO^+$ CLA^+ cells (12.5 vs. 8.5, P = 0.0035); $CD4^+$ $CD45RO^+$ CLA^+ cells (21.2 vs. 12.2, P = 0.000095); and $CD8^+$ $CD45RO^+$ CLA^+ cells (6.1 vs. 1.6, P = 0.000007).

The NI-sensitive subjects were divided into two groups based on cutaneous response following oral exposure (responders N = 13, non-responders N = 20). A dose-response reaction was observed among nickel-sensitive subjects. Both responders and non-responders had significantly higher fractions of $CD3^+$ $CD45RO^+$ CLA^+ lymphocytes before challenge than the healthy controls (P = 0.014 and 0.049, respectively). After challenge this was significant only for the non-responders (P = 0.025). Both Ni-sensitive groups showed significantly higher fractions of $CD4^+$ $CD45RO^+$ CLA^+ cells before and after Ni-challenge (P < 0.001). Responders had the highest fraction of $CD8^+$ $CD45RO^+$ CLA^+ before and after Ni-challenge [7.7 vs. 1.6 (P = 0.022) and 6.5 vs. 1.6 (P = 0.0014), respectively]. Only those individuals that responded to Ni-challenge with 4 mg Ni had significantly elevated levels of IL-5 in the serum (P = 0.025) and a smaller non-significant increase in IL-10. No differences in the levels of IL-2, IL-4, IFN-γ, or TNF-α were observed before or after challenge. Overall the results indicate that $CD8^+$ $CD45RO^+$ CLA^+ T-lymphocytes and T lymphocytes with the type 2 cytokine profile are involved in systemic contact dermatitis associated with nickel exposure.

Minang et al. (2006a) investigated the effect of IL-10 on Ni-induced Th-1(IFN-γ) and Th-2-type (IL-4 and IL-13) cytokine responses in human peripheral blood mononuclear cells

(PBMC). PBMC from 15 Ni-allergic and 8 control donors were stimulated with nickel and the frequency of cytokine-producing cells and cytokine concentrations analyzed by enzyme-linked immunospot (ELISpot) and enzyme-linked immunosorbent assay (ELISA). PBMC suspensions of 2.5 $x10^5$ cells with or without 50 µM $NiCl_2 \cdot 6H_2O$ were incubated with different concentrations of recombinant rIL-10 (0 to 25 ng/mL). Nickel-PBMC showed significantly higher levels of endogenous IL-10 compared to control PBMC. The mean increase in IL-10 induced by Ni(II) was 33.1 pg/mL and 2.2 pg/mL in the Ni-PBMC and control PBMC, respectively. Addition of rIL-10 to Ni-PBMC reduced the levels of Ni-induced IL-13, and IFN-γ. The mean levels of IFN-γ were reduced by 40% to 71% using 0.2 and 1 ng/mL of rIL-10. No effects of rIL-10 were seen in the control PBMC. The results suggest that IL-10 may play a role in vivo in counteracting the allergic reactions mediated by Th-1-type reactions. In a follow-up study the authors observed similar mixed Th1- and Th2-type cytokine profiles in allergic subjects with cobalt(II), chromium(Cr III and VI), palladium(Pd II) and gold(Au I and III). In terms of the optimal dose for induction of cytokines IL-2, IL-4 and IL-13 the order of effectiveness was: Cr(VI), 0.5 µM>; Au(III), 2 µM>; Au(I), 25 µM>; Ni(II) ~ Co(II), 50 µM>; Cr(III) ~ Pd(II), 100 µM.

Caicedo et al. (2007) investigated the metal ion-induced DNA damage, apoptosis, necrosis and proliferation in a human CD4+ T-helper lymphocyte (Jurkat) cell line. Cell suspensions with 1 x 10^6 cells were incubated for 48 hr with 0, 0.05, 0.5, 1.0, or 5.0 mM metal ion. The results indicated that the metal ions did not preferentially induce Jurkat T-lymphocyte DNA damage prior to other forms of toxicity indicated by apoptosis and/or necrosis. In terms of the average concentration (of the four endpoints) required to induce a significant adverse effect, the metals were ranked as follows: V(III), 0.29 mM; Ni(II), 1.41 mM; Co(II), 2.65 mM; Cu(II), >2.65 mM; Nb(V), >2.75 mM; Mo(V), >2.87 mM; Zr(II), >3.875 mM; Be(II), >4 mM; Cr(III), >5 mM; Al(III), >5 mM; and Fe(III), >5 mM. Vanadium (III) and nickel (II) stand out as the more toxic of the metal ions surveyed on average. In terms of cytotoxicity only cobalt (II) and niobium (V) were more toxic (0.5 mM) than vanadium (1.0 mM) and nickel (5.0 mM).

Miyazawa et al. (2008) studied the role of the mitogen-activated protein kinase (MAPK) signaling pathway in the activation of dendritic-type THP-1 cells by nickel sulfate. Nickel and other low molecular weight allergens induce contact hypersensitivity via a cell-mediated delayed-type immune response. In the induction phase these compounds or haptens first make contact with dendritic cells (DCs) in the skin, including Langerhans cells (LCs). Activated DCs migrate to regional lymph nodes and trigger the allergen-specific T-cell response with expression of stimulatory molecules (e.g., CD86 and CD54) and the production of several stimulatory cytokines (e.g., IL-1β). Human myeloid cell lines (THP-1, U937 and MUTZ-3) are good surrogates of DCs and have a high capacity to induce tumor necrosis factor (TNF-ά) release and CD86, CD54 and CD40 expression following allergen treatment. THP-1 cells (1 x 10^6) were cultured for one hour in one mL of culture medium with either 170 µg/mL $NiSO_4$ or 5 µg/mL 2,4-dinitrochlorobenzene (DNCB). Some experiments included the 0.03 to 3 µM of p38 MAPK inhibitor SB203580. Nickel sulfate and DNCB induced phosphorylation of p38 and extracellular signal-regulated kinase (ERK). Inhibition of p38 MAPK activation selectively blocked

DNCB-induced TNF-ά release, but not $NiSO_4$. Alternatively, inhibition of ERK pathways selectively suppressed $NiSO_4$-induced TNF-ά but not DNCB-induced release. The authors conclude that the two allergens activate p38 MAPK and ERK, and stimulate TNF-ά release via different signal transduction pathways.

Boisleve et al. (2005) demonstrated that in immature human $CD34^+$-derived DC, three MAPK pathways (ERK, p38MAPK, and JNK) participated in the expression of CD83, CD86 and CCR7 molecules induced by $NiSO_4$. In contrast, following TNF-α stimulation, only p38 MAPK was involved in CD83 and CCR7 expression. ERK inhibited DC maturation while JNK had no effect. The authors also demonstrated that inhibition of the MAPK pathways did not suppress $NiSO_4$-induced down-regulation of the adhesion molecule E-cadherin and the specific LC protein, langerin, suggesting that other signaling pathways may be involved.

8.2.1 Immunotoxicity Summary

Contact dermatitis is a widespread disease and, in the western hemisphere, nickel sensitization is the most common single cause of contact allergy (Lisby, 1999b). The mechanism underlying nickel-induced allergy is still incompletely understood. As noted in the papers described above most research has focused on T cell activation in Ni-allergic patients. Systemic contact dermatitis in humans has been used to study inflammatory skin disease occasionally seen as a flare-up of previous dermatitis or as de novo dermatitis when sensitized individuals are exposed to the hapten orally, transcutaneously, intravenously or by inhalation. Studies of immunological mechanism of Ni-induced disease have tried to determine if effects are elicited primarily via activation of CD4+ and/or CD8+ T cells of the type 1 or type 2 or even type 0 cytokine profile subsets (Jensen et al., 2004). The likely involvement of MAPK and possibly other signaling pathways in the disease process has added another level of complexity.

8.3 Cytotoxicity

Yoshioka et al. (2007) studied the urinary excretion of 8-hydroxyguanine (8-OH-G), an oxidative stress marker, in nickel-cadmium battery workers. Sixty-six subjects (64 male and two female) provided urine and blood samples. The levels of cadmium in blood (Cd-B) and nickel in urine (Ni-U) were determined by graphite furnace atomic absorption spectroscopy. 8-OH-G in urine was analyzed by high performance liquid chromatography-electrochemical detector system. Creatinine-adjusted 8-OH-G was significantly correlated with age, Ni-U, and Cd-B in univariate analysis, while multivariate analysis revealed that Ni-U and Cd-B were significantly independent variables positively correlated with 8-OH-G. The data were analyzed for mixture toxicity. The subjects were divided into groups based on median concentration of Ni-U and Cd-B (2.86 µg/g creatinine and 0.23 µg/dL, respectively). Subjects with high Ni-U/high Cd-B (Group 4) had the highest levels of 8-OH-G (21.7±2.0, GM±GSD), followed by those with high Ni-U/low Cd-B (11.5±1.6, Group 3), those with low Ni-U/high Cd-B (Group 2, 8.9±1.9) and those with low Ni-U/low Cd-B (Group 1, 8.5±1.5). The p values of Student's t-tests between Group 1 and Group 2, 3, and 4 were 0.847, 0.050, and < 0.001, respectively. The combined effect of Cd and Ni on the urinary

excretion of 8-OH-G departed from additivity. The results indicate that nickel exposure was the primary stressor resulting in increased production and excretion of 8-OH-G.

Carroll and Wood (2000) exposed monolayer cultures of human keratinocytes and fibroblasts to nickel sulfate at concentrations above 0.001 M. Cytotoxicity to both cell types was 50% based on decreased viability. ^{35}S-methionine labeling followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with specific monoclonal antibodies indicated an increased synthesis of heat shock protein 90 (Hsp90) in keratinocytes at concentrations above 10^{-5} M and induction of heat shock protein 72 (Hsp72) above 10^{-4} M. For fibroblasts increased induction of Hsp90 was seen at all concentrations tested and a dose-related increase was observed for Hsp72. The results indicate a stress response to the toxic effects of nickel ions at fairly low concentrations.

Cell lines derived from monkey kidney (COS-7), human lung tumors (A549), or human liver tumors (HepG2) were cultured for four days with 0, 100, 200, or 400 µM Ni Cl_2. Nickel treatment decreased growth rates in all cell lines after four days in a dose dependent manner. In HepG2 cells GRP96 expression was significantly enhanced at 400 µM Ni(II) ($P < 0.05$) whereas Hsp72 and Hsp73 were significantly suppressed ($P < 0.01$). COS-7 cells showed a similar pattern. GRP96 was over-expressed in A549 cells at 400 µM Ni(II) and Hsp73 was moderately increased.

Au et al. (2006) studied the cytotoxicity of nickel(II) in human T-lymphocyte Jurkat cells in vitro. Jurkat cells were incubated with 0, 1, 10, or 100 µg/mL Ni^{2+} (compound unspecified) for 24 hours. The treatment reduced cell viability and proliferation in a dose-dependent manner. Cell viability was reduced by 35% at 100 µg Ni/mL. A significant decrease in cell proliferation was also seen at 100 µg Ni/mL. Nickel(II) at 10 µg Ni/mL induced expression of caspase-3, but not at 100 µg Ni/mL. Cells incubated at 100 µg Ni/mL showed fragmented nuclei. Enumeration of Hoechst 33258-stained cells showed that Ni^{2+} at 100 µg/mL induced 16% of the cells to undergo apoptosis. In contrast the lower Ni concentrations were indistinguishable from the control. The authors note that the onset of apoptosis by metal ions may be due to a disruption in cell signaling, DNA damage, or changes in cell constituents such as Ca^{2+}.

M'Bemba-Meka et al. (2006) exposed isolated human lymphocytes to solubilized Ni_3S_2 in vitro to assess cytotoxicity. Lymphocyte suspensions were exposed to 0, 0.25, 0.50, 0.75, 1.0, 1.5, or 2.0 mM Ni_3S_2 for 3-4 hr and to 2.0 mM Ni_3S_2 for 30, 60, 90, 120, 180 or 240 min. Cell viability was assessed by trypan blue exclusion. Nickel(II) treatment resulted in both concentration- and time-dependent lymphocyte death. Significant increases in cell death were seen at 0.75 mM Ni_3S_2 for 4 hr and 1.0 mM Ni_3S_2 for 2 hr ($P < 0.05$). Increased production of H_2O_2 and superoxide anion (O_2^-), lipid peroxidation and depletion of cellular sulfhydryl contents were induced by 1 mM Ni_3S_2. Nickel-induced lymphocyte death was significantly prevented by pretreatment with scavengers of reactive oxygen species (catalase, superoxide dismutase, dimethylthiourea/mannitol, deferoxamine or glutathione/*N*-acetylcysteine). Co-treatment with cyclosporin A inhibited Ni_3S_2-induced disturbances of mitochondrial membrane potential ($\Delta\Psi m$), and

significantly prevented Ni_3S_2-induced cell death ($P < 0.05$ vs. Ni_3S_2 alone treatment). Lymphocyte death was also significantly reduced by treatment with Ca^{2+} channel blockers (diltiazem, nifedipine, and verapamil) and intracellular Ca^{2+} antagonists (dantrolene, cyclosporin A, and ruthenium red). Treatment of lymphocytes with 1 mM Ni_3S_2 alone increased intracellular Ca^{2+} about three fold over three hours. The authors interpret the findings as indicative of an activation of cell death signaling pathways involving generation of reactive oxygen species (ROS) and oxidative stress, loss of mitochondrial membrane potential, and disruption of cellular calcium homeostasis.

Guan et al. (2007) also studied the toxicity of nickel(II) in human T-lymphocyte Jurkat cell line. The cells were exposed to 0, 20, 40, 60, or 80 µg Ni/mL $NiCl_2$ for 0, 6, 12, or 24 hr and viability measured by trypan blue staining assay. Viability was less than 10% when cells were incubated for 24 hr at 80 µg Ni/mL. Treated cells exhibited morphological changes and chromosomal condensation indicative of apoptosis. The apoptotic fraction increased in a dose- and time-dependent manner. After incubation with nickel(II) for 6 hr the concentration of NO increased linearly from ca. 0.9 (control) to 3.7 µM (80 µg Ni/mL) (monitored by release of NO_2^-/NO_3^- into the cell culture medium). Nickel(II) treatment was also observed to dissipate mitochondrial membrane potential and down regulate bcl-2 mRNA after 12 hr exposure at 60 µg Ni/mL possibly modulating Ni-induced cell apoptosis. The authors speculate that a key process in the immune cellular response to nickel(II) is nickel induced apoptosis mediated by a mitochondrial pathway associated with NO.

Ke et al. (2007) studied fluorescent tracking of nickel ions in human cultured cells. Water-insoluble nickel compounds such as NiS and Ni_3S_2 were shown in vitro to enter cells by phagocytosis. Using a dye that fluoresces when intracellular Ni^{2+} ion binds to it, the authors showed that both soluble and insoluble nickel compounds elevated Ni ions in the cytoplasm and nuclear compartments. However, soluble nickel compounds were more readily removed than the insoluble nickel compounds. Within 10 hours after $NiCl_2$ removal from the culture medium, Ni ions disappeared from the nucleus and were not detected in the cells by 16 hours. Insoluble Ni_3S_2 yielded Ni ions that persisted in the nucleus after 16 hours and were detected in the cytoplasm even after 24 hours following Ni removal.

Trombetta et al. (2005) evaluated the toxic effects of nickel in a three dimensional model of human epithelium (RHE) reconstituted from TR146 cells derived from a human squamous cell carcinoma of the buccal mucosa. The RHE cultures were exposed for 72 hr to eight concentrations of $NiCl_2$ ranging from 0.05 to 7.6 mM. Cell viability, assessed by the MTT assay, was significantly reduced at Ni(II) concentrations greater than 1.3 mM. Similarly the release of prostaglandin E2 and interleukin-6 into the culture medium was also significantly increased above 1.3 mM Ni(II). However no change was seen in interleukin-8 release at any nickel concentration. In addition to cytokines the effect of nickel on glutathione (GSH) was also measured. Nickel induced a non statistically significant reduction in GSH from 2.392 nmol/cm^2 in control cultures to 2.151 nmol/cm^2 at 7.6 mM Ni(II). By contrast an increase in tissue oxidized glutathione (GSSG) was seen at all nickel concentrations and was statistically significant above 0.7 mM ($P <$

0.05). Total tissue glutathione (GSH + GSSG) appeared to increase compared to controls after nickel exposure. The ratio of GSH/GSSG was significantly reduced at all nickel concentrations tested ($P < 0.05$). The results indicate that nickel exposures that are not toxic enough to affect cell viability or inflammatory cytokine release can affect cellular redox equilibrium. The authors also observed an increase in vacuolized cells and apoptotic cells in tissue cultures at all Ni concentrations ≤ 0.7 mM without evidence of cellular necrosis. Thus low "non-toxic" nickel exposure may modify cellular effectors of apoptosis.

Davidson et al. (2005) reported that $^{63}NiCl_2$ interfered with cellular iron homeostasis in human lung A549 cell cultures. Soluble nickel was observed to block the uptake of iron into transferrin-bound iron and non-transferrin-bound iron (NTBI) leading to cellular ferritin accumulation. Since excessive iron is toxic to cells, such nickel-induced blockage might be expected to lead to cytotoxicity. Nickel also decreased the binding of Von Hippel-Landau (VHL) protein to hypoxia inducible factor 1α (HIF-1α) possibly by competing for iron sites on prolyl hydroxylases. Prolyl hydroxylases 1-3 hydroxylate the ODD (oxygen-dependent degradation) domain in HIF-1α. VHL can bind to hydroxylated proline residues in the ODD domain of HIF-1α and target it for degradation. When the prolyl hydroxylases are not functional, no hydroxylation of proline residues occurs and VHL will not bind.

Cheng et al. (2003) quantified gene expression in microarrays with cDNA chips (ca. 8000 cDNAs) after exposure of human peripheral lung epithelial cells to nickel(II). Cultured human lung epithelial HPL1D cells were exposed for 24 hr to non-cytotoxic (50, 100, or 200 µM) or cytotoxic (400, 800, or 1600 µM) Ni^{2+} concentrations. Cytotoxicity was assessed by loss of cell adhesion in 70% confluent cultures after 24 hr Ni-exposure. The data set comprising 868 genes was filtered to select only those 113 genes, which showed a $\geq$ 2-fold change in expression at one or more of the three nontoxic nickel concentrations. Most of the genes impacted by low nickel concentrations were related to gene transcription, protein synthesis and stability, cytoskeleton, signaling, metabolism, cell membrane, and extracellular matrix.

Gazel et al. (2008) evaluated transcriptional profiles in Ni(II) treated human epidermal keratinocytes using DNA microarrays. Reconstructed human epidermis (RHE) was exposed to 11 µM $NiSO_4$ for 30 min or 6 hr. Microarray analysis showed that 134 genes were affected by Ni(II) exposure: 97 genes were induced and 37 genes were suppressed. The functional categories of affected genes indicated that Ni(II) inhibits apoptosis, promotes cell cycle and induces synthesis of extracellular matrix proteins and proteases. Ni also regulates secreted signaling proteins, inducing vascular endothelial growth factor (VEGF), amphiregulin (AREG), placental growth factor (PGF), prostate differentiation factor (GDF15), and bone marrow stromal cell antigen 2 (BST2), while suppressing IL-18, galectin-3 (LGALS3), and lipopolysaccharide-induced TNF-α Factor (LITAF). Interestingly no Ni(II) effects were seen in epidermal differentiation genes.

Ouyang et al. (2009) studied the effect of nickel compounds on the cell cycle in human lung carcinoma A549 cells in vitro. $NiCl_2$ at doses from 0.25 to 1.0 mM were found

equivalent to 0.25 to 2 μg NiS/cm^2 in the activation of transcription factor NFkB and HIF-1α, and induction of TNF-α and CAP43 gene expression. Growth of A549 cells was significantly inhibited by 0.25 mM $NiCl_2$ but only marginally inhibited by NiS at 2.0 μg/cm^2. Nickel sulfide also failed to significantly inhibit human bronchial epithelial cell line HCCBE-3 or mouse skin epidermal cell line C141. Exposure to $NiCl_2$, but not NiS, caused a significant inhibition of cell growth and G1/G0 cell cycle arrest concomitant with a marked down-regulation of cyclin D1 in A549 cells. The down-regulation is due to protein degradation rather than inhibition of transcription. The degradation of cyclin D1 is a ubiquitination- and proteosome-dependent process, but how soluble nickel initiates or regulates this process is unknown. Effects on other cell cycle regulatory proteins were also evaluated namely,.cyclin E and p21. Nickel had no effect on cyclin E while both nickel compounds increased the amounts of p21.

Rossman (2009) has criticized the use of dyes, particularly Trypan Blue in the assessment of cytotoxicity when used close to the time of exposure. These methods give better results (close to results with clonal survival) when used about three days after exposure; otherwise cytotoxicity may be significantly underestimated.

8.3.1 Epigenetic effects

The effects of nickel(II) on the epigenome are summarized in Table 5A. Effects on DNA methylation and/or histone methylation, acetylation, or ubiquitination may influence the initiation and/or progression of chronic diseases in addition to cancer.

Zhang et al. (2003) observed inhibition and reversal of nickel-induced transformation by the histone deacetylase inhibitor trichostatin A (TSA). Human T85 osteoblastic cells (HOS) were exposed to 0, 0.15, or 0.30 μg/cm^2 Ni_3S_2 or 0, 1, 2 mM $NiCl_2$ for 24 hr. The cells were rinsed, allowed to grow for 48 hr and the Ni treatment repeated. This procedure was repeated nine times. Either 5.0 ng/mL or 25 ng/mL TSA were added to the cells four hr before each exposure. Ni treated HOS cells exhibited dose-dependent increases in anchorage-independent colonies with both nickel compounds (ca. 500-750/10^5 cells vs. 250/10^5 cells in controls). Similar exposure to mouse PW cells showed 150 -250/10^5 cells for $NiCl_2$ and 1500-2200/10^5 cells for Ni_3S_2 vs. 0 for controls. TSA treatment caused a dose-dependent suppression of Ni-induced transformation of HOS and PW cells. For HOS cells treated with 2 mM $NiCl_2$ the extent of transformation at 0, 5.0, and 25.0 ng/mL TSA was 100%, 59.5% ($P < 0.05$), and 51.0% ($P < 0.01$), respectively. For HOS cells treated with 0.30 μg/cm^2 Ni_3S_2 the extent of transformation was 100%, 93.3% and 78.9% ($P<0.05$), respectively. Suppression was greater in the mouse PW cells (range 67 to 39%). Isolated Ni-transformed clones of mouse PW cells were reverted to normal by treatment with 5.0 ng/mL or 25.0 ng/mL TSA. Transformed cells ranged from 33 to 65% at 5 ng TSA/mL, 16 to 36% at 25.0 ng TSA/mL vs. 100% in untreated Ni-transformed clones.

Ke et al. (2006) studied alterations of histone modifications and transgene silencing by $NiCl_2$. Human lung bronchoepithelial A549 cells in culture were exposed to 0, 0.25, 0.50, or 1.0 mM $NiCl_2$ for 24 hr. Using pan-acetylated histone antibodies, the global

levels of histone acetylation on histones H2A, H2B, H3 and H4 were measured following nickel exposure. The nickel doses had no effect on cell viability whereas histone acetylation was decreased in all four-core histones. A similar loss of histone acetylation was also observed in human hepatoma Hep3B cells, mouse epidermal C141 cells and *gpt* transgenic Chinese hamster G12 cells. Nickel treatment also resulted in increases of ubiquitination of H2A and H2B in a dose-dependent manner. The G12 *gpt* transgenic cell line was used to measure Ni-induced gene silencing in cells treated for 7 to 21 days with 50 or 100 μM $NiCl_2$ or 1 μg/cm^2 NiS. Treatments of three days or longer resulted in increased frequency of 6-thioguanine (6-TG) resistant colonies suggesting silencing of the *gpt* transgene in a time-dependent manner. After Ni-treatment the cells were placed in normal medium for either one or five weeks. The mRNA levels of the *gpt* transgene, which were very low after Ni treatment, returned to basal level after five weeks recovery. The data suggest that the nickel-induced effects were epigenetic.

Chen et al. (2006) reported that $NiCl_2$ treatment of human lung carcinoma A549 cells induced increases in histone H3 lysine 9 dimethylation and transgene silencing. Nickel(II) ions were found to increase global histone H3K9 mono- and dimethylation but not trimethylation. Increases in dimethylation occurred at ≥ 250 μM Ni(II) in a time-dependent manner. Nickel exposure decreased the activity of histone H3K9 methyltransferase G9a thus interfering with the histone dimethylation process. Cultured transgenic gpt^+ $hprt^-$ G12 cells were used to study Ni-induced gene silencing. Both acute and chronic nickel exposures decreased the expression of the *gpt* transgene in G12 cells. The cells were exposed to Ni(II) for 3 to 25 days to 50 or 100 μM $NiCl_2$ then selected for the gpt^- phenotype by growing cells in the presence of 6-thioguanine (6-TG). Nickel exposure increased the frequency of $6\text{-}TG^r$ variants in a dose- and time-dependent manner. The variants were treated with 5-aza-2'-deoxycytidine resulting in a very high percentage reversion from gpt^- to gpt^+ phenotype. Such a high frequency of reversion indicates that Ni(II) silenced the *gpt* transgene via an epigenetic rather than a genetic mechanism involving mutations or deletions. Overall the results indicated that the increase in H3K3 dimethylation played a key role in the *gpt* transgene silencing due to Ni(II) exposure.

Karaczyn et al. (2006) observed that human lung cells treated with Ni(II) resulted in a stimulation of mono-ubiquitination of H2A and H2B histones. Cultured 1HAEo and HPL1D human diploid lung cells were treated for 1 to 5 days with 0.05 to 0.5 mM Ni(II) acetate. Cell viability, assessed by Trypan blue exclusion, ranged from 90% at the low nickel concentration to 55-65% at the high concentration. Maximum stimulation of ubiquitination of H2B histone was reached in 24 hr at 0.25 mM Ni(II) in both cell lines. The authors note: "covalent modifications of core histones in chromatin, such as acetylation, methylation, phosphorylation, ribosylation, ubiquitination, sumoylation, and possibly others (e.g. deimination and biotinylation) serve as regulatory mechanisms of gene transcription." Usually increased ubiquitination of histone H2B is associated with gene silencing and decreased ubiquitination with gene activation, although this may depend on gene location. The authors interpret their results on Ni-induced histone ubiquitination as part of nickel's adverse effects on gene expression and DNA repair.

Ji et al. (2007) investigated epigenetic alterations in a set of DNA repair genes in NiS-treated 16HBE human bronchial epithelial cells (0, 0.25, 0.5, 1.0, or 2.0 µg Ni/cm^2 for 24 hr). The silencing of the O^6-methylguanine DNA methyltransferase (MGMT) gene locus and upregulation of DNA methyltransferase 1 (DNMT1) expression was observed in treated cells. Other epigenetic alterations included DNA hypermethylation, reduced histone H4 acetylation and a decrease in the ratio of Lys-9 acetylated/methylated histone H3 at the MGMT CpG island in NiS-transformed 16HBE cells. It's likely that Ni-induced alterations in DNA and histones contribute to altered gene expression, cytotoxicity and tumorigenicity.

Ke et al. (2008) demonstrated the both water-soluble and insoluble nickel compounds induce histone ubiquitination (uH2A and uH2B) in a variety of cell lines. Human A529 lung cells were treated with $NiCl_2$ (0.25, 0.5, and 1.0 mM) or Ni_3S_2 (0.5 and 1.0 µg/cm^2) for 24 hr. After exposures histones were isolated and Western blots performed using antibody against uH2A. $NiCl_2$ and Ni_3S_2 exposures resulted in increased levels of uH2A in a dose-dependent manner. Other mouse and human cell line tested were C141, Beas-2B, HeLa, and Hep3B. In each case $NiCl_2$ treatment resulted in increased levels of uH2A. In vitro assays indicated that the presence of nickel did not affect the levels of ubiquinated histones through increased synthesis; instead nickel significantly prevented loss of uH2A and uH2B presumably inhibiting putative deubiquitinating enzyme(s). The study indicates that nickel ions may alter epigenetic homeostasis in cells.

Li et al. (2009) studied signaling pathways induced by nickel in non-tumorigenic human bronchial epithelial Beas-2B cells. Both 0.25 mM and 1.0 mM $NiSO_4$ exposure for 24 hr significantly up-regulated *c-Myc* protein in Beas-2B cells in a time-dependent manner. Because of the central role of *c-Myc* in cell growth regulation, cell apoptosis was also studied. Beas-2B cells were treated with $NiSO_4$ and whole cell lysates to determine poly (ADP-ribose) polymerase (PARP) cleavage, a marker for cell apoptosis. Nickel ions at 0.5 and 1.0 mM significantly induced PARP cleavage, indicating $NiSO_4$- induced apoptosis in the Beas-2B cells. Knockout of *c-Myc* and its restoration in a rat cell system confirmed the role of *c-Myc* in Ni(II)-induced apoptosis. Ni(II) ions increased the *c-Myc* mRNA concentration and *c-Myc* promoter activity but not *c-Myc* mRNA and protein stability. By the use of pathway specific inhibitors the investigators concluded that Ni(II) induced *c-Myc* in Beas-2B cells via the *Ras/ERK* signaling pathway. The study suggests possible roles for *c-Myc* in Ni-induced toxicity.

The effects of nickel, chromate, and arsenite on histone 3 lysine 4 (H3K4) methylation in human A549 cells was evaluated by Zhou et al. (2009). Treatment of human lung carcinoma A549 cells with Ni(II) (1.0 mM), Cr(VI) (10 µM), or As(III) (1.0 µM) significantly increased tri-methyl H3K4 after 24 hr exposure. Seven days exposure to lower levels (e.g., 50 µM Ni(II)) also increased tri-methyl H3K4. The results indicate that the metals studied alter various histone tail modifications which can affect the expression of genes that may cause cell transformation or other cytotoxic effects. The specific genes that may be affected by these alterations are unknown. Other relevant DNA methylation and mapping of post-translational modifications of histones in the promoter regions of target genes warrant further investigation.

Table 5A. Effects of Nickel(II) on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Li et al. 2009	$NiSO_4$	c-Myc c-Myc	↑ ↑	BEAS-2B HaCaT	Apoptosis induced
Guan et al. 2007	$NiCl_2$	bcl-2	↓	T cells Jurkat	Apoptosis induced, NO ↑
Andrew & Barchowsky, 2000	Ni_3S_2	PAI-1	↑	BEAS-2B	Fibrinolysis inhibited
Andrew et al., 2001	Ni_3S_2	PAI-1 c-Jun c-Fos	↑ ↑ ↑	BEAS-2B	Fibrinolysis inhibited
Salnikow et al., 2002	$NiCl_2$	HIF-1α *Cap43* *Nip3* *Prolyl-4-hydroxylase* *HSP70* *GADD45* *p21* *p53*	↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑	Mouse fibroblasts HIF-1α knockout	Hypoxia, *Nip3* and prolyl-4-hydroxylase are HIF-1 dependent; *HSP70*, *GADD45*, *p21* and *p53* are HIF-1 independent; *ATM, GADD153, Jun B* and *MDR-1* are mixed.
Salnikow et al., 2003	$NiCl_2$	HIF-1α *Cap43* *Bcl-2* *Nip3* *EGLN1* *HIG1* *Prolyl-4-hydroxylase* *Focal adhesion kinase*	↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑	Mouse fibroblasts HIF-1α knockout	HIF-1 independent genes up-regulated *GADD45, p21, ATM, p53, Jun B;* genes up-regulated in HiF-1α deficient cells *HSP70, NGFb, IP-10, CD44 antigen, melanocortin 1 receptor, heparin-binding EGF-like, SGK kinase, BCL-2-like, E-MAP-115.*
Davidson et al., 2003	$NiCl_2$	HIF-1α AhR *CYP1B1* *NQO1* *UDP glucuronyltransferase 1A6*	↑ ↓ ↓ ↓ ↓	Mouse fibroblasts HIF-1α knockout	All genes suppressed were HIF-independent including *prostaglandin endoperoxide synthase I* and *glutathione S – transferases μ, α3,* and *α Ya*

Table 5A. Effects of Nickel(II) on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Li et al., 2004	$NiCl_2$ Ni_3S_2	HIF-1α *Cap43* protein expression	↑	Mouse C141 epidermal cells and PI-3K and Akt deficient mutants	Activation of phosphatidylinositol 3-kinase (PI-3L), Akt, and p70S6 kinase ($p70^{S6k}$).
Broday et al., 2000	$NiCl_2$ Ni_3S_2	Histone H4 acetylation	↓	A549 lung carcinoma cells, yeast cells	Lysine 12 acetylation in H4 inhibited in A549 cells and at Lys 12, 16, 5, and 8 in yeast.
Yan et al., 2003	Ni_3S_2 NiS	*gpt+* gene silencing	↓	G12 Chinese hamster transgenic *gpt+* cells	Histones H3 and H4 hypoacetylated,H3K 9 methylated, H3K9 deacetylated
Ke et al., 2006; 2008	$NiCl_2$ NiS	*gpt+* gpt^-	↓	A549 cells, Hep3B cells, G12 Chinese hamster transgenic *gpt+* cells, and gpt^- clones N24, N37, N96	Histones H2A, H2B, H3 and H4 deacetylated, increases of H3K9 dimethylation, increases of H2A and H2B ubiquitination, minimal cytotoxicity. Ni acts by inhibiting deubiquitination
Chen et al., 2006	$NiCl_2$	*gpt+* gene silencing	↓	G12 Chinese hamster transgenic *gpt+* cells, A549 cells	Increased mono- and dimethylation of histone H3K9, decreased H3K9 methyltransferase G9a. gpt silencing reversed by dimethylation of H3K9 with 5-aza-2'-deoxycytidine

Table 5A. Effects of Nickel(II) on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Zhang et al., 2003	Ni_3S_2 $NiCl_2$	Reversion of Ni-induced cell transformation	↑	Human HOS TE85 cells, mouse PW cells	Cells treated with histone deacetylase inhibitor trichostatin A (TSA) had increased frequency of revertants in transformed cells
Ji et al., 2008	NiS	*MGMT* *DNMT1*	↓ ↑	NiS-transformed human 16HBE cells	Silencing of *MGMT* associated with DNA hypermethylation, altered histones H3K9me2, H4ac and H3K9ac, and *DNMT1* upregulation.
Karaczyn et al., 2006	Ni(II) counter ion unspecified	Dysregulation of H2B ubiquitination	↑	1HAEo- and HPL1D human lung cells	Histone H2B and H2A ubiquitination stimulated by Ni(II) exposure. H2B was monoubiquinated and H2A mono- and diubiquinated.
Kang et al., 2003	$NiCl_2$	Histone acetylation Reactive oxygen species (ROS)	↓	Human Hep3B hepatoma cells	Dose- and time-dependent decrease in H4 acetylation. Ni(II) inhibited histone acetyltransferase (HAT) but not histone deacetylase (HDAC). ROS involved in MOA.
Zhou et al., 2009	$NiCl_2$	Histone methylation	↓ ↓	A549 cells NHBE cells	H3K4 increased di- and tri-methylation but not mono-methylation.

*Note: BEAS, human bronchial epithelial cells; HaCaT, human keratinocyte cells; NO, nitric oxide generation; PAI-1, plasminogen activator inhibitor-1; HIF-1α, hypoxia-inducible transcription factor-1; AhR, aryl hydrocarbon receptor; A549, human lung bronchoepithelial cells; H3B, human hepatoma cells; H3K9, histone H3 lysine 9; HOS, human osteoblastic cell line; PW, mouse embryo fibroblasts; *MGMT*, O^6-methylguanine DNA methyltransferase gene

locus; *DNMT1*, DNA methyltransferase 1 gene; 16HBE, Ni-transformed human bronchial epithelial cells; H3K9ac, histone H3 lys-9 acetylation; H4ac, histone H4 acetylation; H3K9me2, histone H3 Lys-9 methylation; NHBE, normal human bronchial epithelial cells; ↑, enhanced activity; ↓, reduced activity.

8.4 Genetic Toxicity

While genetic toxicology generally provides key supporting documentation for cancer risk assessment rather than the present noncancer assessment, we believe that mutagenicity and other genetox effects contribute to chronic disease and ageing irrespective of their role in initiation and promotion of tumors (F.M.Burnet, 1974). In particular nickel's effects on the epigenome and gene expression indicate the probability that nickel genetic toxicity is relevant to its noncancer effects.

The International Agency for Cancer Research (IARC, 1990), the International Program on Chemical Safety (IPCS, 1991), and NTP (1998) have reviewed the genotoxicity of nickel and nickel compounds in humans. Waksvik and Boysen (1982) studied groups of nickel refinery workers (9-11 workers in each group) and observed increases in chromosomal aberrations compared to controls. Deng et al. (1988) found elevated levels of both sister chromatid exchanges and chromosome aberrations (gaps, breaks, fragments) in seven electroplating workers exposed to nickel and chromium. Kiilunen et al. (1997) found that the frequency of micronucleated epithelial cells in the buccal mucosa of nickel refinery workers in the Helsinki area was not significantly elevated versus controls. The significance of these study results is somewhat limited due to the small sample sizes and the possibility that some workers were exposed to genotoxic compounds other than nickel. We summarize genetic toxicity findings in human test systems in Table 5B.

Table 5B. Genotoxicity of Nickel Compounds in Human Test Systems (adapted from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Nickel chloride	Human diploid fibroblasts	DNA damage	-	Hamilton-Koch et al., 1986
Nickel sulfate	Human gastric mucosal cells	DNA damage	-	Pool-Zobel et al., 1994
Nickel chloride	Human HeLa cells	DNA replication	+	Chin et al., 1994
Nickel sulfate Nickel sulfide	Human lymphocytes	Sister chromatid exchange	+	Andersen, 1983; Larremendy et al., 1981; Ohno et al., 1982; Saxholm et al., 1981
Nickel sulfate	Human lymphocytes	Chromosome aberration	+	Larremendy et al., 1981
Nickel subsulfide	Human lymphocytes	Sister chromatid exchange, metaphase analysis, micronuclei formation	+ + +	Arrouijal et al., 1982
Nickel sulfate	Human bronchial epithelial cells	Chromosome aberration	+	Lechner et al., 1984
Nickel subsulfide Nickel oxide Nickel sulfate Nickel acetate	Human foreskin cells	Cell transformation	+	Bidermann and Landolph,1987
Nickel oxide Nickel subsulfide Nickel carbonate hydroxide nickel sulfate	Human lymphocytes	Sister chromatid exchange	- +	Waksvik and Boysen, 1982; M'Bemba-Meka et al. 2007
Nickel chloride	Human lymphocytes	DNA strand breakage, Comet assay	+	Chen et al., 2003
Nickel containing particles	Human A549 lung cells	Cytotoxicity, DNA repair capacity, mutation frequency	+	Mehta et al., 2008

Chen et al. (2003) evaluated the effects of nickel chloride on genotoxicity in human lymphocytes in vitro. Peripheral blood mononuclear cells (PBMC, primarily lymphocytes) were collected from five randomly selected healthy individuals (aged 18 to 23). Isolated lymphocytes (2×10^6 cells/µL) were incubated in saline solution with 0, 0.5, 1.0, 2.5, 5.0, or 10.0 mM $NiCl_2$ for one hour at 37ºC with continuous shaking in the dark. The levels of intracellular reactive oxygen species (ROS), lipid peroxidation, hydroxyl radical (•OH), and DNA damage via the Comet assay were evaluated.

The viability of the lymphocytes based on either trypan blue or neutral red exclusion decreased in a dose-dependent manner (neutral red control 92.3 % vs. 69.7% at 10 mM $NiCl_2$). Intracellular oxidants measured by dichlorofluorescin (DFC) increased in a dose-dependent manner (control 4.8% vs. 59.9% fluorescence intensity at 10 mM $NiCl_2$) with all dose levels significantly greater than the control. 2-Thiobarbituric acid reactant substances (TBARS) were also significantly increased compared to control at all $NiCl_2$ levels (control 156.5 vs. 553.7 nmol/10^6 cells at 10 mM $NiCl_2$). Lipid peroxidation in lymphocytes was significantly increased by three-fold with 10 mM $NiCl_2$. Hydroxy radical production was measured by the hydroxylation of salicylate to 2,3-dihydroxybenzoate (2,3-DHB) and 2,5-dihydroxybenzoate (2, 5-DHB) byproducts. Both byproducts were significantly increased by $NiCl_2$ in a dose-dependent manner. The greater increase was seen with 2, 3-DHB (control 33.3 vs. 80.5 nM/10^6 cells at 10 mM $NiCl_2$). DNA damage as assessed by the extent of cell tailing in the Comet assay was increased in a dose-dependent manner (control 60 vs. 260 arbitrary units at 10 mM $NiCl_2$). The authors conclude that the generation of •OH radical was responsible for the $NiCl_2$-induced DNA strand breakage as evidenced by the dose-dependent association with •OH radical generation and comet tailing. The high correlation of DNA damage and DHB byproducts ($r^2 = 0.9519$) indicates that ROS in Ni-treated lymphocytes are responsible for Ni-induced oxidative stress. The generation of Ni-induced •OH radical may play an important role in genotoxicity in human cells.

Broday et al. (2000) observed nickel-induced inhibition of histone H4 acetylation in yeast and human cells in vitro. *Saccharomyces cerevisiae* cells were grown in medium with 0, 0.2 or 0.5 mM $NiCl_2$ for 1, 3, or 6 cell generations. Histones were isolated and analyzed with antibodies specific for H4 acetyl-lysine 5, 8, 12, or 16. The addition of 0.5 mM $NiCl_2$ suppressed the growth-related accumulation of lysine acetylation at all four lysine residues compared with the control cells. The effect of nickel on the levels of histone acetylation was also examined in human lung carcinoma A549 cells treated with soluble $NiCl_2$ and insoluble Ni_3S_2. The soluble nickel treatment of 0 or 3 mM $NiCl_2$ did not change the level of H4 acetylation. Nickel subsulfide treatment at 0, 0.5, 1.0 µg/cm^2 for two days (40 to 80% confluent growth) resulted in a concentration-dependent decrease in H4 acetylation at Lys-12. The concentrations used were reported as nontoxic. What toxic effects may result from altered histone acetylation patterns in vivo, particularly when coupled with Ni-induced DNA methylation, is unknown.

Jia and Chen (2008) studied nickel-induced DNA damage and cell death in human leukemia HL-60 cells and the protecting role of antioxidants. Cells were treated for up to 96 hr with 0, 0.5, 1.0, or 10.0 mM Ni^{2+}. Ten mM Ni^{2+} was rapidly fatal to cells along

with a concomitant increase in DNA fragmentation as measured by flow cytometry with propidium iodide. Lower concentrations of Ni^{2+} also resulted in DNA fragmentation and death but at lower levels and after much longer exposures, i.e. no less than 48 or 72 hr at 1.0 or 0.5 mM, respectively. Nickel treatment of HL-60 cells also resulted in a release of malondialdehyde (MDA) in a dose- and time-dependent manner. The antioxidants ascorbic acid and *N*-acetyl-cysteine significantly reduced the Ni-induced generation of MDA and DNA fragmentation in a dose-dependent manner. Alternatively, H_2O_2 increased both Ni-induced MDA generation and DNA fragmentation also in a dose-dependent manner. Similar results were obtained for the cell death endpoint.

Mehta et al. (2008) evaluated the effects of particulate matter containing nickel and chromium on nucleotide excision repair capacity (NER) in human lung cells in vitro. They observed that human A549 cells exposed to 100 µg/mL of urban particulate matter (collected in the Washington DC area) for 24 hr had only a 10% reduction in viability, but a 35% reduction in repair capacity, and a five-fold increase in mutation frequency. The authors interpret their results with a view to three potential mechanisms: (1) particle components such as heavy metals and aldehydes directly modify repair proteins and DNA; (2) ROS and secondary products of ROS modify repair proteins and DNA; and (3) direct modification of DNA replication proteins by heavy metals and aldehydes reduce the fidelity of DNA replication. Specifically "Ni and cadmium can induce repair protein-DNA damage complex formation. … Aldehydes, Cr, and Ni are known to have a high affinity towards thiol groups and histones and, therefore, their potential targets could be zinc finger structures in DNA binding motifs."

9. Chronic Toxicity to Experimental Animals

9.1 Pneumotoxicity

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

Tanaka et al. (1988) exposed male Wistar rats (five/dose group) to green NiO aerosols (MMAD = 0.6 µm) for 7 hr/day, 5 days/week for up to 12 months. The average exposure concentration was either 0.3 mg/m^3 or 1.2 mg/m^3. For histopathological examination, rats were sacrificed at 3, 6, and 12 months of exposure and 8 months following a 12-month exposure. The nickel content of rat lungs was up to 2.6 mg and 0.6 mg after 12 months exposure at the high and low concentrations, respectively. Higher incidence of lesions in exposed compared to control animals was seen for pneumonia in all exposure durations at low and/or high exposure concentrations and for bronchiolar metaplasia and adenomatosis for 12 months exposure at the low and/or high exposure concentrations.

A two-year inhalation study of nickel oxide in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (0, 0.5, 1.0, or 2.0 mg Ni/m^3) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all

exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m^3 or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m^3 for both sexes, and in males exposed to 1.0 mg Ni/m^3.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m^3. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m^3 group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 μg Ni/m^3 as nickel oxide for two years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were observed in the animals exposed to nickel. Only one rat per group survived the nickel exposures to the end of the experiment.

A two-year study on the effects of nickel subsulfide in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg Ni_3S_2/m^3 (0, 0.11, or 0.73 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg Ni/m^3 compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy were observed in both sexes exposed to 0.73 mg Ni/m^3.

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg Ni_3S_2/m^3 (0, 0.44, or 0.88 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg Ni/m^3 and the 0.88 mg Ni/m^3 concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

An exposure of rats to either 0 or 0.97 mg Ni_3S_2/m^3 (0 or 0.71 mg Ni/m^3) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs (Ottolenghi *et al.*, 1974).

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg $NiSO_4/m^3$ (0, 0.03, 0.06, or 0.11 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks.

Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg Ni/m^3 or greater.

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg Ni/m^3 as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg Ni/m^3 or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg Ni/m^3.

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide six hours/day, five days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg Ni/m^3 or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as low as 0.02 mg Ni/m^3. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m^3.

Obone et al. (1999) evaluated the effects of 0, 44.7, 111.75, or 223.5 mg Ni/L in drinking water of male Sprague-Dawley rats exposed for 13 weeks. Alkaline phosphatase activity in bronchoalveolar lavage fluid (BALF) was significantly decreased at all dose levels compared to the control animals (8/dose group, $P < 0.05$). No significant changes were seen in the activities of alkaline phosphatase, acid phosphatase, or lactate dehydrogenase in lung tissues after 13 weeks exposure. However, a significant increase in BALF proteins was seen at 111.75 and 223.5 mg Ni/L $NiSO_4$ in drinking water ($P<0.05$).

McDowell et al. (2000) exposed C57BL/6 mice to $NiSO_4 \cdot 6H_2O$ aerosol in a steel inhalation chamber. The particulate aerosol had a MMAD of 0.22 µm and a gsd of 1.85 µm with a chamber concentration of 110 ± 26 µg/m^3. The mice were exposed for 0 (control), 3, 8, 24, 48, or 96 hr before sacrifice and assessment of the progression of lung injury by microarray analysis with murine complimentary DNAs. Lung polyadenylated mRNA was isolated, reverse transcribed, and fluorescently labeled. Samples from exposed mice (Cy5 labeled) were competitively hybridized against samples from unexposed, control mice (Cy3 labeled) to microarrays containing 8734 murine cDNAs. Of the > 8700 genes analyzed, 17 were differentially expressed at 3 hr and 255 at 96 hr. The overall pattern of gene expression with increasing lung injury was indicative of oxidative stress, hypoxia, cell proliferation and extracellular matrix repair, followed by a decrease in surfactant proteins.

Oller et al. (2008) evaluated the effects of inhaled nickel metal powder in a chronic study in Wistar rats. The animals (50/sex/dose group) were exposed by whole-body inhalation to 0, 0.1, 0.4 and 1.0 mg Ni/m^3 nickel metal powder (MMAD = 1.8 µm, gsd = 2.4 µm) for six hr/day, five days/week for up to 24 months. High mortality in the 1.0 mg Ni/m^3 dose group resulted in earlier termination of exposures in this group. No NOAEL was observed. Non-respiratory treatment-related histopathological lesions were a granular brown pigment in the kidneys, extramedullary hematopoiesis in the spleen and hypercellularity of sternum and femoral bone marrows, all in both sexes. Respiratory tract lesions included alveolar proteinosis, alveolar histiocytosis, chronic inflammation, bronchiolar-alveolar hyperplasia and bronchial lymph node infiltrate. Nearly all of these effects exhibited dose-responses in both sexes.

A benchmark dose analysis of the data in Oller et al. (2008, Table 5B) for the sum of moderate and severe incidences of respiratory tract lesions is summarized in Table 6. $BMDL_{05}$ values ranged from 1 to 12 µg Ni/m^3. A similar analysis of non-respiratory tract lesions (not shown) gave $BMDL_{05}$ values ranging from 8 µg Ni/m^3 (female spleen) to 27 µg Ni/m^3 (male kidney). An average dosimetric adjustment factor (DAF) of 0.395 was derived from Multipath Particle Deposition (MPPD) model (v.2) airway deposition calculations for the rat and average of human age groups (3 months to 21 years) exposed continuously to 0.1 mg Ni/m^3. The human equivalent concentration (HEC) is calculated as Rat Concentration x DAF.

At the 78-week evaluation significant increases ($P < 0.01$) were seen in mean red blood cell count (RBC), hemoglobin levels (Hb) and hematocrit values (HCT) at 0.1 and 0.4 mg Ni/m^3 in males and at 0.4 mg Ni/m^3 in females. These findings were suggested by the study authors as possibly resulting from hypoxia secondary to lung injury, however, they note that similar increases were seen in another study of oral nickel sulfate hexahydrate exposure when no lung injury was observed (Heim et al., 2007). Also, a direct effect of nickel on gene expression of erythropoietin has been reported (e.g. Salnikow et al. 2000). A continuous benchmark dose analysis was conducted on the blood effects data (Oller et al., 2008, Table 3). For male rats the $BMDL_{1SD}$ values for RBC, Hb and HCT averaged 1.9 µg/m^3 and, for females, averaged 3.1 µg/m^3. All the individual data sets were well fit visually by the polynomial model although there were insufficient degrees of freedom to do a fitness test (data not shown).

Ogami et al. (2009) evaluated the toxicity of different sizes of nickel oxide particles following intratracheal instillation in rats. Two sizes of NiO were used: a fine sized NiO with a median diameter on 0.8 µm (nNiOm), and micrometer sized NiO with a median diameter of 4.8 µm (NiO). The particle distributions were bimodal (NiO) or trimodal (NiO) with lower or higher peaks than the median, respectively. The pathological effects were compared with crystalline silica (SiO_2, geometric mean diameter 1.6 µm, gsd = 2.0) and TiO_2 (geometric mean diameter 1.5 µm, gsd = 1.8) particles. The particles (2.0 mg) were suspended in 0.4 mL saline and instilled into Wistar rats (10 weeks old, 25 animals/group) along with a saline only control group. Animals were sacrificed at three days, one week, one month, three and six months after particle instillation. At autopsy 50 mL of bronchoalveolar lavage fluid (BALF) were obtained by injecting saline into the

right lung of each animal. Total cells and polymorphonuclear leukocytes (PMN) in BALF were recovered and counted.

The number of total cells in BALF in the nNiOm group was significantly higher than the control and the other particle treatments at all time periods except SiO_2 at 6 mo when comparable values were seen (all $P < 0.01$). NiO showed a gradual increase in total cells with a significant difference at 6 mo. ($P < 0.05$). The PMN percentages in BALF were significantly higher than controls for nNiOm and SiO_2 for all time periods, although nNiOm decreased over time (40% to10%) while SiO_2 increased (40% to 65%) (all $P < 0.01$). TiO_2 also showed a significant increase at three days only (25%, $P < 0.05$). The inflammation area rate by the point counting method showed a gradual increase for nNiOm with significant increases vs. controls at all time points with a peak at 3 mo ($P < 0.01$). SiO_2 also increased gradually showing the highest value at 6 mo ($P < 0.01$). No significant differences were seen for the NiO or TiO_2 groups. The results suggest that submicrometer nano-nickel oxide is significantly more toxic to the lung than micrometer-sized nickel oxide. The observed effects were similar in qualitative and quantitative respects to those caused by similar administration of crystalline silica but apparently less persistent.

Lu et al. (2009b) evaluated several short-term in vitro assays for predicting the potential of metal oxide nanoparticles including NiO to cause pulmonary inflammation. The assays were intrinsic free radical generation, extracellular oxidative activity, cytotoxicity to lung epithelial cells, hemolysis, and inflammation in rat lungs via intratracheal instillation. Twelve nanoparticles (NPs) ranging from 2-4 nm (Al_2O_3, alumina 1) to 300 nm (Alumina 3) were included in the study. The nickel oxide was characterized as 10-20 nm in size, 92 m^2/g in surface area, and 5.4 mg/500 cm^2 in mass. Intrinsic free radical generation (IFR) was assessed by electron paramagnetic resonance with surface area doses of 1,500 and 3,000 cm^2/mL. Only NiO, CeO_2, Co_3O_4 and carbon black (CB) showed significant increases in IFR over control ($P < 0.05$). Oxidative potential was measured with a cell-free dichlorofluorescein assay and significant fluorescence intensity over control was observed only for NiO, Co_3O_4, and CB ($P< 0.05$). Cytotoxicity was assessed by incubating alveolar A549 cells with NPs at different surface area doses (9.4 – 300 cm^2/mL) for 24 hr and measuring lactate dehydrogenase (LDH) release in cell lysates. There were clear positive LDH dose-responses for NiO, Co_3O_4 and CB. Linear dose-dependent hemolytic activity in fresh human venous blood was observed for NiO, CeO_2, and alumina 2. Lung inflammation in vivo was assessed by intratracheal instillation of NPs at 500 cm^2/mL in rats and measuring polymorphonuclear neutrophils (PMN) numbers in BALF 24 hr after instillation. Only NiO and alumina 2 were significantly inflammogenic at the dose employed. Of the assays evaluated, only blood hemolysis gave a correct prediction of lung inflammatory activity for 12/13 NPs (CeO_2, false positive). NiO gave the strongest positive response in all five assays and gave the largest inflammation response *in vivo* (total PMN).

Table 6. Benchmark Dose Analysis of Respiratory Tract Lesions Induced by Nickel Metal Inhalation in Wistar Rats (Data of Oller et al. 2008).*

Lung Lesion Observed	Incidence at 0, 0.1, 0.4 mg/m^3	X^2	P	BMD_{05} mg/m^3	$BMDL_{05}$ mg/m^3	$BMDL_{05}$ µg/m^3
Male						
Proteinosis	0/50, 19/50, 40/50	0.35	0.83	0.012	0.0095	1.7
Histiocytosis	0/50, 7/50, 17/50	0.69	0.71	0.045	0.0326	5.8
Inflammation	0/50, 1/50, 22/50	0.34	0.84	0.12	0.07	12.5
Hyperplasia	1/50, 3/50, 9/50	0	1.0	0.12	0.069	12.3
Lymph node infiltrate	0/34, 4/37, 9/42	1.16	0.56	0.073	0.0475	8.5
Female						
Proteinosis	0/50, 22/50, 38/54	0	1.0	0.0077	0.0053	0.95
Inflammation	0/50, 10/50, 23/54	0	1.0	0.021	0.012	2.1
Lymph node infiltrate	0/39, 4/42, 9/44	0.88	0.64	0.078	0.051	9.1

*Note: All dose responses fit with the multistage-quadratic model of BMDS v 1.4.1c; values are for rats adjusted for continuous exposure (values multiplied by 6/24 x 5/7) but not for human equivalent concentrations.

9.2 Immunotoxicity

Some of the immunologic effects of nickel in exposed rodents in vivo are summarized in Table 7. Smialowicz et al. (1984, 1985) injected nickel chloride i.m. in mice and found a significant reduction in a variety of T-lymphocytes and natural killer cell-mediated immune functions. They also demonstrated that suppression of natural killer cell activity could be detected with *in vitro* and *in vivo* assays and that reduction of natural killer cell activity was not associated with either a reduction in spleen cellularity or the production of suppressor cells. Their findings confirmed those reported by other investigators on the immunosuppressive effects of nickel compounds on circulating antibody titers to T_1 phage in rats (Figoni and Treagan, 1975), on antibody response to sheep erythrocytes (Graham et al., 1975), on interferon production in vivo in mice (Grainer et al., 1977), and on the susceptibility to induced pulmonary infection in mice following inhalation of nickel chloride (Adkins et al., 1979).

Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m^3 as Ni_3S_2; 0.47, 2.0, or 7.9 mg Ni/m^3 as NiO; and 0.027, 0.11, and 0.45 mg Ni/m^3 as $NiSO_4$ for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m^3 as nickel subsulfide. In contrast, AFC responses in

the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m^3 as nickel chloride 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

Obone et al. (1999) evaluated the bioaccumulation and toxicity of nickel sulfate in rats following 13 weeks of oral exposure. Adult male Sprague-Dawley rats (8/dose group) were given 0, 0.02%, 0.05% and 0.1% nickel sulfate, i.e. 0, 44.7, 111.75, and 223.5 mg Ni/L, in their drinking water for 13 weeks. Measurements of splenic lymphocyte subpopulations following exposure to 0.05% $NiSO_4$ showed significant increases in absolute numbers of T-cells, CD4+ and CD8+. Statistically significant increases in CD8+ and decrease in the ratio of CD4/CD8 were observed at all dose levels. Significant increases in both the absolute number and percentage of thymocyte CD8+ cell populations were also seen at all dose levels. The findings indicate a LOAEL of approximately 7.0 mg/kg-d for immunotoxicity ($C = 0.1*W^{0.7377}$ L/d, W = 0.185 kg rats; U.S.EPA, 1988).

Harkin et al. (2003) studied immunosuppression in Sprague-Dawley rats following i.p. administration of 0 (vehicle), 0.12, 0.36, 1.1, or 3.3 mg $NiCl_2$/kg bw. Nickel chloride suppressed T-lymphocyte proliferation and Th-1 (IFN-γ) and Th-2 (IL-10) cytokine production in a dose- and time-dependent manner. In addition, $NiCl_2$ inhibited production of the pro-inflammatory cytokine TNF-ά and increased the production of the anti-inflammatory cytokine IL-10 from lipopolysaccharide (LPS) stimulated cultures. Three of the cytokine data sets from Harkin et al. (2003) were subjected to continuous benchmark dose analysis (their Figure 2 (a), (b), and (c)). All the data sets fit the Hill model with P values greater than 0.22 ($P \geq 0.1$ adequacy of fit criterion). For concanavalin-A (Con-A) stimulated Th1:IFN-γ, the $BMDL_{1SD}$ was 0.18 mg Ni^{2+}/kg bw. For Con A-stimulated Th2:IL-10, the $BMDL_{1SD}$ was 0.14 mg Ni^{2+}/kg bw. LPS-stimulated TNF-ά gave a $BMDL_{1SD}$ of 0.17 mg Ni^{2+}/kg bw. The similarity of the quantitative dose responses for nickel-induced cytokine suppression may indicate a common mode of action. The authors reported that the minimum plasma concentrations of nickel required to provoke immunosuppression are in the range 209 to 585 ng/mL. In the kinetic portion of the study a 3.3 mg/kg $NiCl_2$ dose provoked immunological changes that were maximal one hour following administration. The data demonstrate that $NiCl_2$ suppresses T-cell function and promotes an immunosuppressive macrophage phenotype in rats.

Table 7. Immunologic Effects of Nickel Compounds Observed in Rodent Studies (NTP, 1996a)

Nickel Compound	Species/Route	Chemical treatment	Response	Reference
Cell-mediated immunity				
Nickel chloride	CBA/J mice, intramuscular	Single injection, 18 mg/kg bw	Reduced T-lymphocyte proliferation	Smialowicz et al., 1984
Nickel sulfate	B6C3F1 mice female, oral	Up to 4,000 mg/kg-d for 23 weeks	Depressed spleen lymphoproliferative response to LPS (no effect on NK activity; PFC assay; mitogen response in spleen cells; resistance to *Listeria* challenge)	Dieter et al., 1988
Nickel sulfate	Sprague-Dawley rats, oral, drinking water 13 weeks	0, 0.02, 0.05, 0.1%$NiSO_4$•$6H_2O$, or 0, 44.7, 11.75, 223.5 mg Ni/L	Increase of CD4+ and CD8+ T-cells and decrease of CD4/CD8 ratio	Obone et al. 1999.
Humoral immunity				
Nickel chloride	CBA/J mice, intramuscular	Single injection, 18 mg/kg bw	Reduced antibody response to T-cell dependent sheep red blood cells	Smialowicz et al., 1984
	Swiss albino mice, intramuscular	3-12 µg Ni/kg bw followed by immunization with sheep red blood cells	Depressed antibody formation	Graham et al., 1975
	Swiss mice, inhalation	2-hour inhalation exposure at 250 µg/m3	Depressed antibody response to sheep red blood cells	Graham et al., 1978
Nickel acetate	Sprague-Dawley rats, intraperitoneal	11 mg/kg bw immunized with *E. coli* bacteriophage	Depressed circulating antibody response	Figoni and Treagan, 1975
Macrophage function				
Nickel chloride	CBA/J mice, intramuscular	Single injection, 18 mg/kg bw	No effect on phagocytic capacity of peritoneal macrophages	Smialowicz et al., 1984

Table 7. Immunologic Effects of Nickel Compounds Observed in Rodent Studies (NTP, 1996a)

Nickel Compound	Species/Route	Chemical treatment	Response	Reference
Natural killer cell activity				
Nickel chloride	CBA/J and C57BL/6J mice, intramuscular	Single injection, 18 mg/kg bw	Depressed NK activity against Yac-1 murine lymphoma cells	Smialowicz et al., 1984, 1985, 1986
Host resistance				
Nickel chloride and nickel oxide	CD mice and Sprague-Dawley rats, inhalation	0.5 mg/m^3 for 2 hours	Enhanced respiratory infection by *Streptococcus*	Adkins et al., 1979

Roberts et al. (2009) studied the metal components of residual oil fly ash (ROFA) on pulmonary host defense in rats. The soluble fraction of ROFA contained Ni, Fe, Al and Zn. Sprague-Dawley rats were intratracheally instilled with 55.7 µg/rat ($NiCl_2$), 32.7 µg/rat ($FeSO_4$), 46.6 µg/rat ($Al_3(SO_4)_2$, 8.69 µg/rat ($ZnCl_2$), or a combination of all metals. Rats were also instilled with mixtures without a specific metal e.g., Mix-No Ni. Prior to infection with *Listeria monocytogenes* (5×10^4 cells) soluble nickel alone or in metal mixture produced no more lung injury than saline controls. Following infection nickel-treated animals had increased bacterial lung burden and body weight decrease. Ni alone and in mixtures increased reactive oxidants in the lung and was most important in suppressing T-cell activity following infection. Weight decreases in the mixes without Fe or Al indicate that iron and aluminum may act antagonistically to nickel. Overall the authors conclude that soluble Ni is the primary metal involved in the increased susceptibility to infection observed in rats exposed to the soluble metals of ROFA.

9.3 Cytotoxicity

Morimoto et al. (1995) studied the effects of nickel oxide (green) on the production of tumor necrosis factor (TNF) by alveolar macrophages of rats exposed in vitro and in vivo. For in vivo exposure five male Wistar rats (nine weeks old) were exposed to 11.7 ± 2.0 mg NiO/m^3 for 8 hr/day, 5days/week, for 4 weeks along with five unexposed control animals. Bronchoalveolar lavage was performed and recovered alveolar macrophages were assayed for TNF production. Nickel oxide exposure produced a three-fold higher concentration of TNF produced by macrophages from exposed animals compared to controls ($P < 0.01$). In addition acid phosphatase and lactate dehydrogenase (LDH) release from macrophages were also significantly greater ($P<0.01$) than controls, both indicators of cytotoxicity.

Shiao et al. (1998) investigated the effects of nickel acetate on cell cycle, apoptosis and p53 expression in Chinese hamster ovary (CHO) cells in vitro. CHO cells were grown for 72 hours in medium containing 0, 40, 80, 160, 240, 320, 480, or 640 µM nickel(II)

acetate. DNA fragmentation, representative of apoptosis, was examined by gel electrophoresis. The distribution of cells in various stages of the cell cycle was determined by DNA flow cytometry and p53 expression by the Western blotting technique. DNA fragmentation was seen at nickel concentrations ≥ 160 µM. The proportion of cells at S-phase declined in a Ni^{2+} concentration-dependent manner above 160 µM (33% to 12%). The decline was accompanied by an increase in the proportion of G_2/M phase cells (9% to 26%). Expression of p53 was not affected by nickel exposure. The authors conclude that these cellular responses were most likely induced by a common effector(s) that cause G_2/M arrest and concurrent apoptosis. P53 protein is apparently not responsible for the effects seen but nickel(II) up-regulates other proteins, which may be involved.

Gurley et al. (1983) studied the toxicity to CHO cells in vitro of particulate Ni_5As_2, one of a number of nickel arsenides formed during oil shale retorting. The insoluble Ni_5As_2 powder was suspended with the cells at concentrations of 0, 10, 25, 50, and 100 µM. At 10 µM Ni_5As_2 the growth rate doubling time was increased from 16.5 hr (control) to 40 hr. At 100 µM Ni_5As_2 growth was completely inhibited. Cell cycle analysis showed that Ni_5As_2 concentrations ≥ 50µM cells accumulated in the G2 +M phases. Cells treated for 24 hr with 25 µM Ni_5As_2 and transferred to nickel arsenide free medium completely recovered viability but grew at a slower than control rate. Cells similarly treated at 50 or 75 µM nickel arsenide had survivals of only 61% and 25%, respectively.

Takahashi et al. (1999) studied the cytotoxicity of two types of NiO (black and green) and five intermediate types prepared by calcinations of black NiO at 600-1000ºC. The NiO forms varied in Ni and O content, color and X-ray diffractometric pattern. They also varied in water solubility from NiO(B) at 6-7µg/mL to 1-3 µg/mL for calcined forms and 0.5-1.5 for NiO(G). Cytotoxicity was assessed with rat alveolar macrophages obtained from female Sprague-Dawley rats aged 12-16 weeks and CHO cells cultured in vitro. The viability of rat alveolar macrophages exposed to NiO at 800 µg/mL for 18, 42 and 72 hr showed the greatest toxicity for NiO(B) followed by NiO(600ºC) and NiO(800ºC). CHO cells exposed to 50, 100, or 200 µg/mL of each nickel oxide for 24 hr exhibited a dose and compound related decrease in cell proliferation from NiO(B) to NiO(G) with the calcined forms in order of temperature. The authors conclude that water solubility which is inversely related to calcination temperature modulates the cytotoxicity of NiO particles.

Clemens and Landolph (2003) evaluated the cytotoxicity and cell transformation of mouse embryo cells by samples of nickel refinery dust containing different concentrations of nickel arsenide and pure nickel arsenide. Mouse embryo C3H/T101/2 cells (200/dose) were treated with 0, 0.5, 1.0, 2.5, 5.0 or 7.5 µg/mL. The dust samples were composed largely of NiO and $Cu_2Ni_8O_{10}$ with 25% Ni_5As_2 in dust sample 1 and 2.5% Ni_5As_2 in dust sample 2. After treatment for 48 hr the cells were recovered and assayed for survival. For each treatment the average survival fraction was plotted to determine the 50 percent lethal concentration (LC_{50}) value. Dust sample 1 and nickel arsenide gave identical LC_{50} values of 2.4 µg/mL, whereas dust sample 2 with less Ni_5As_2 gave a slightly lower LC_{50} of 1.7 µg/mL. Although the dust sample appeared to

be more cytotoxic than the other samples, the reverse was true in parallel chromosome aberration and cell transformation assays.

Nickel chloride induced lactate dehydrogenase (LDH) release and lipid peroxidation (LPO) in rat renal cortical slices in vitro in a concentration- (0 to 2.0 mM) and time- (0 to 4 hr) dependent manner (Chakrabarti and Bai, 1999). Both $NiCl_2$-induced LDH release and LPO were significantly prevented by glutathione and dithiothreitol, suggesting that $NiCl_2$-induced renal cell injury is partially dependent on thiols. Superoxide dismutase partially reduced the $NiCl_2$-induced LDH release without affecting LPO and glutathione, whereas catalase did not affect such LDH release and LPO. Dimethylthiourea and DMSO completely prevented $NiCl_2$-induced LPO, but only partially reduced LDH release. Deferoxamine prevented $NiCl_2$-induced renal cell injury without affecting LPO and without significantly reducing Ni^{2+} uptake by the renal cortex, suggesting that nickel chelation is not important in prevention of cell injury. $NiCl_2$-induced loss of cellular glutathione was significantly prevented by thiols and deferoxamine, but not by superoxide dismutase or dimethylthiourea. The results suggest that LPO was not related to $NiCl_2$-induced lethal renal cell injury. Renal cell injury was more likely the result of the induction of the Fenton reaction, generating hydroxyl radicals.

The effects of nickel chloride on the expression patterns of stress proteins in rat organs and human and monkey cell lines was studied by Hfaiedh et al. (2005). Three-month old female Wistar rats were injected i.p. with 4 mg $NiCl_2$/kg bw for 1, 3, 5, or 10 days. Rat kidneys, liver and ovaries were cut into small pieces, sonicated briefly in lysis buffer, and 5000 x g (30 min) supernatants collected and frozen until use. Relative protein expression in total organ extracts was measured for three proteins, namely, cytosolic Hsp72 and Hsp73, and the reticulum-associated GRP94. In kidney, nickel induced significant increases ($P < 0.01$) in GRP96 and Hsp73 at ≥ 3 days of treatment (GRP96) and at 3 and 5 days (Hsp73). Hsp72 was significantly suppressed at all days of treatment ($P < 0.05$). Few effects were noted in liver or ovary. Dietary restriction (1 month 50%) did not significantly alter the results. The authors infer that Ni-induced GRP94 over-expression in kidney and in cell lines could be mediated by hypoxic stress at the cellular level.

The effects of nickel ions on reductive amination and oxidative deamination activities of bovine liver glutamate dehydrogenase (GDH) were studied kinetically by UV spectroscopy (Ghobadi et al., 2007). The fact that Ni^{2+} ions have the capacity to enhance binding of NADH(reduced nicotinamide adenine dinucleotide) to the enzyme was confirmed by an electrochemical method. Ni^{2+} decreased the Km for NADH from 0.083 mM (control) to 0.053 mM at 200 µM $NiCl_2$. The NADPH (reduced nicotinamide adenine dinucleotide phosphate) Km was similarly decreased (0.077 to 0.036 mM, respectively). Lineweaver-Burk plots with respect to alpha-ketoglutarate and ammonium ions indicated substrate and competitive inhibition patterns in the presence of nickel ion, respectively. Adenosine diphosphate (ADP) at 0.2 mM protected inhibition caused by nickel. The observations are explained by the authors in terms of formation of a nickel-NADH complex with a higher affinity for binding to the regulatory site in GDH, than in the absence of nickel.

Lu et al. (2009) studied the mechanisms of cytotoxicity of Ni(II) ions based on gene expression profiles. Mouse fibroblast cells (L-929) were cultured in medium with 0, 100, 200, 300, 400, or 500 µM $NiCl_2 \cdot 6H_2O$ for 24, 48, or 72 hours. Cytotoxicity was assessed by methylthiazoltetrazolium (MTT) assay. Ni-induced cytotoxicity was dose- and time-dependent. After 72 hr, cell viability was reduced from 100% (control) to 36.1% at 500 µM. Gene expression was assessed by cDNA microarray analysis of cells treated with 200 µM Ni(II) for 24, 48, or 72 hr. Twenty up-regulated and 19 down-regulated genes were differentially expressed in all three exposure periods. Gene ontology analysis showed that the Ni- affected genes represented biological processes (e.g., development-7%, cellular process-36%, physiological process-38%), molecular function (e.g., binding-52%, catalytic activity-24%, signal transducer-6%), and cellular components (cell-48%, protein complex-8%, organelle-36%). Specifically the down-regulation of the *Hsp90aa1* gene affected the processes associated with cell adhesion, cell morphogenesis, regulation of cell proliferation, and regulation of cell migration. Overall the results showed broad effects on gene expression even when no obvious cytotoxicity was evident (i.e., 91.5% viability at 200 µM Ni(II), 24 hr). Ni(II) has extensive effects on cells by inhibiting cell proliferation and differentiation, through inducing cell apoptosis, affecting cell development and influencing cholesterol metabolism.

9.3.1 Epigenetic effects

Salnikow et al. (2002) studied gene expression in nickel(II) treated mouse embryo fibroblasts with and without the hypoxia-inducible transcription factor-1 (HIF $-1\alpha^{+/+}$, HIF-$1\alpha^{-/-}$). HIF-1α strongly induces hypoxia-inducible genes, including the tumor marker gene *Cap43*. The wild type and knockout cells were exposed to 1.0 mM $NiCl_2$ for 20 hr and gene expression assessed by cRNA hybridization and GeneChip microarray analysis. Nickel exposure induced genes involved in glucose metabolism in HIF-1α-proficient cells. Of 12 glycolytic enzyme genes studied by microarray 10 were induced by Ni(II) exposure in proficient but not in HIF-1α deficient cells. Glucose-6 phosphate dehydrogenase and hexokinase I were the only unaffected genes. Nickel(II) was also found to induce some genes in HIF-1α proficient and deficient cells (*HSP70, GADD45, p21, p53, ATM, GADD, JunB,* and *MDR-1).*

In a subsequent study, Salnikow et al. (2003) found a number of genes induced by Ni(II) in HIF-1α deficient but not in proficient cells. Among these genes are *NGF-β, SGK, IP10, CD44*, heparin binding EGF-like, melanocortin 1 receptor, *Grg1*, *BCL-2*-like, and tubulin-binding protein *E-Map-115*. IFN-inducible protein 10 (IP10) is a chemokine that targets T cells and NK cells. The elevation of *IP10* expression has been demonstrated in human diseases including chronic cirrhosis and biliary atresia (Koniaris et al., 2001). Most of the nickel-induced genes appear to be related to stress response. A number of genes were significantly suppressed by nickel exposure in an HIF-1-dependent manner (i.e. suppression was greater in HIF-1α proficient cells compared with HIF-1α deficient cells) including monocytes chemoattractant protein 1 (*MCP-1*) and the tumor suppressor gene *Zac1*. *Zac1* induces apoptosis and cell cycle arrest and was not suppressed in HIF-1α deficient cells. Neuropilin-1 (*Npn-1*) was also suppressed by nickel in an HIF-1α-dependent manner. Neuropilin is a transmembrane receptor in endothelial and other

cells. The effects of nickel on gene expression after 20 hr exposure were transient and disappeared after nickel removal, although chronic nickel exposure can lead to selection of cells in which these changes persist.

Salnikow et al. (2003) evaluated the modulation of gene expression by $NiCl_2$ and Ni_3S_2 in two mouse and one human cell lines. Mouse embryo fibroblast cell lines MEF-HIF1α and PW were exposed to 0, 0.03, 0.1, 0.3, 1.0, or 2.0 µg Ni_3S_2/cm^2 or 0, 0.125, 0.25, 0.5, 1.0, or 2.0 mM $NiCl_2$ for 20 hr. Total RNA was isolated from Ni-exposed and control cells and cDNA prepared for GeneChip analysis. Both soluble and insoluble nickel compounds induced similar signaling pathways in the mouse cell lines. The microarray data indicated increases in expression of genes involved in glucose metabolism including glucose transporter I and glycolytic enzymes such as hexokinase II, phosphofructokinase, pyruvate kinase, and triosephosphate and glucose phosphate isomerases and lactate dehydrogenase. All of these genes are induced by hypoxia, suggesting that nickel similarly induces the HIF-1 transcription factor, which regulates these genes. Other HIF-1 genes induced included Tdd5, Egln I, Nip3, Est and Gly96. The results indicate that the form of nickel has little effect on the Ni-induced alterations of gene expression and are expected to have similar carcinogenic or other toxic potential in vivo.

Davidson et al. (2003) studied the interaction of the aryl hydrocarbon receptor (AhR) pathway and the hypoxia inducible factor-1α (HIF-1α) pathway in nickel-exposed cells. HIF-1α knockout and wild type cells were derived from C57B mice. Mouse cells exposed to1.0 mM $NiCl_2$ for 24 hr exhibited the suppression of several AhR-regulated genes including CYP1B1, NQO1, UDP-glucuronyltransferase 1A6, and glutathione *S*-transferase Ya. All of the observed AhR-dependent genes except glutathione *S*-transferase θ1 were down regulated in the HIF-1α knockout cells. The most suppressed gene was CYP1B1, which was reduced 22.9-fold in wild type cells and 29.7-fold in knockout cells. Desferrioxamine and hypoxia were also able to suppress basal and inducible expression levels of AhR genes. Dimethyloxalylglycine, an inhibitor of Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenases also inhibited AhR-dependent gene expression in an HIF-1α-dependent manner. The authors conclude that an Fe(II)-, 2-OG- or oxygen-dependent enzyme may be involved in the regulation of AhR-dependent transcriptional activity by nickel(II).

Yan et al. (2003) studied histone modifications and gene silencing in nickel-treated *gpt* transgenic G12 Chinese hamster cells. Four nickel-induced gpt-silenced G12 clones (N24, N37, N96, N97) obtained by treatment with NiS or Ni_3S_2 were used. These clones were readily reverted to wild type (gpt^+) by treatment with 5-azacytadine. Analysis of chromatin proteins associated with Ni-silenced *gpt* gene was by chromosome immunoprecipitation assay (ChIP). The results showed hypoacetylation of both histones H3 and H4 in all four silenced G12 cell clones. Histone H4 acetylation of N24 was higher than the other clones but much lower than G12 control cells. The ChIP assay also showed hypoacetylation of histone H3-K9 in all four silenced clones. Alternatively, methylation was higher than controls in three of four silenced clones. Overall the results indicate that gene silencing induced by nickel involved the loss of histone acetylation and

the activation of histone methylation. Silenced clones exhibited an increase in the methylation of the lysine 9 in histone H3.

Lee (2006) studied differential gene expression in nickel(II)-treated normal rat kidney cells. NRK-52E cells were exposed for two months to 0, 160 and 240 µM Ni^{2+} (acetate). cDNAs corresponding to mRNAs for which expression levels were altered by nickel were isolated, sequenced and followed by GenBank Blast homology search. Specificity of differential expression of cDNAs was determined by reverse transcriptase-polymerase chain reaction. Two of the nickel(II) responsive differential display clones were down regulated: SH3 glutamic acid-rich protein (SH3BGRL3) and fragile histidine triad (FHIT). One clone was up-regulated, metallothionein. The expression of these mRNAs was nickel concentration-dependent. The author notes that SH3BGRL3 probably belongs to the thioredoxin-like superfamily. These small disulfide-reducing enzymes act as hydrogen donors and are thought to be involved in regenerating glutathionated proteins. Down-regulation of SH3BGRL3 may be related to apoptotic death of NRK-52E cells induced by nickel (e.g., as noted by Shiao et al., 1998). Metallothionein is involved in the regulation of physiologically important trace metals such as copper and the detoxification of toxic metals. Since the kidney is a target organ of nickel toxicity the observed up-regulation of metallothionein is not surprising.

9.4 Genetic Toxicity

The Agency for Toxic Substances and Disease Registry (ATSDR, 2005), NTP (1998), Snow (1992), Kasprzak (1991), IPCS (1991), Costa (1991), IARC (1990), the California Air Resources Board (CARB, 1991), and Sunderman (1989) have reviewed the genotoxicity data and mode of action of nickel and nickel compounds. In Table 8 are summarized the *in vitro* and *in vivo* genotoxicity data of nickel compounds in microbial and mammalian test systems. In general the data suggest that nickel does not alter the frequency of gene mutations in non-mammalian systems although some studies have found gene mutations (ATSDR, 2005). The results in mammalian systems are stronger with increased gene mutations found at the HGPRT locus in Chinese hamster V79 cells (Hartwig and Beyermann, 1989; Miyaki et al., 1979) but not in Chinese hamster ovary (CHO) cells (Hsie et al., 1979). Increased gene mutations were also seen in CHO AS52 cells (*grp* locus) (Fletcher et al., 1994), mouse lymphoma cells (Amacher and Paillet, 1980; McGregor et al., 1988), and virus-infected mouse sarcoma cells (Biggart and Murphy, 1988; Biggart et al., 1987).

Table 8. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Microbial systems				
Nickel chloride Nickel nitrate Nickel sulfate	*Salmonella typhimurium*	Gene mutation	-	Arlauskas et al., 1985; Biggart and Costa, 1986; Marzin and Phi, 1985;Wong, 1988
Nickel chloride	*Escherichia coli*	Gene mutation	-	Green et al., 1976
Nickel chloride	*Escherichia coli*	DNA replication	+	Chin et al., 1976
Nickel chloride	*Corynebacterium* sp.	Gene mutation	+	Pikalek and Necasek, 1983
Nickel oxide Nickel trioxide	*Bacillus subtilis*	DNA damage	-	Kanematsu et al., 1980
Nickel chloride	*Saccharomyces cerevisiae*	Histone H4 acetylation decreases at Lys5,8,12,16	+	Broday et al., 2000
Mammalian systems				
Nickel chloride	CHO cells	Gene mutation at the HGPRT locus	-	Hsie et al., 1979
Nickel chloride	Virus-infected mouse cells	Gene mutation	+	Biggart and Murphy, 1988; Biggart et al., 1987
Nickel chloride Nickel sulfate	Mouse lymphoma cells	Gene mutation	+	Amacher and Paillet, 1980; McGregor et al., 1988
Nickel chloride	Chinese hamster V79 cells	Gene mutation	+	Hartwig and Beyersmann, 1989; Miyaki et al., 1979
Nickel chloride Crystalline NiS	CHO cells	DNA damage	+	Hamilton-Koch et al., 1986; Patierno and Costa, 1985
NiO (black and green) NiS (amorphous) Nickel subsulfide Nickel chloride Nickel sulfate Nickel acetate	CHO AS52 cells	Gene mutation (*grp* locus)	+	Fletcher et al., 1994
Nickel chloride Nickel sulfate NiS (crystalline)	Hamster cells	SCE	+	Andersen, 1983; Larremendy et al., 1981; Ohno et al., 1982; Saxholm et al., 1981

Table 8. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Nickel sulfate Nickel chloride NiS	Hamster cells	Chromosome aberration	+	Conway and Costa, 1989; Larremendy et al., 1981; Sen and Costa, 1986b; Sen et al., 1987
Nickel sulfate	Rat bone marrow and spermatogonia cells	Chromosome aberration	-	Mathur et al., 1978
Nickel chloride Nickel sulfate Nickel nitrate	Mouse bone marrow cells	Micronucleus test (oral)	+	Sobti and Gill, 1989
Nickel chloride	Mouse bone marrow cells	Chromosome aberrations (i.p.)	-	Dhir et al., 1991
Nickel chloride	Mouse bone marrow cells	Micronucleus test (i.p.)	-	Deknudt and Leonard, 1982
Nickel acetate	Mouse	Dominant lethal test (i.p.)	-	Deknudt and Leonard, 1982
Nickel subsulfide	Human lung fibroblast MRC-5 cells	DNA strand breaks, PADPRP activation	+ +	Zhuang et al., 1996
Nickel chloride	CHO Cells	DNA repair inhibition	+	Lynn et al. 1997; Iwitzki et al. 1998
Nickel subsulfide	Transgenic mouse	Gene mutation (inhalation)	-	Mayer et al., 1998
Nickel subsulfide	Rat	Gene mutation respiratory tissue (inhalation)	-	Mayer et al., 1998
Nickel sulfide Nickel chloride Nickel sulfate	BALB/c-3T3 Ni-transformed cells in vitro	DNA strand breaks (comet), DNA-protein crosslinks, Telomerase	+ + +	Lei et al. 2001
Nickel sulfate	Chinese hamster V79 cells	Gene mutation, Chromosome aberrations, Aneuploidy, Polyploidy	+ + + +	Ohshima, 2003

Table 8. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Nickel sulfate	Human lung tumor cell lines, HCC15, NCI-H2009, A427	Induction of microsatellite mutations	+ + +	Zienolddiny et al., 2000
Nickel subsulfide	Human lung carcinoma A549 cells	Histone H4 acetylation decrease at Lys 12	+	Broday et al., 2000
Nickel chloride	Male Mice	Dominant lethal mutation	+	Doreswamy et al., 2004
Nickel chloride	Male Mice	DNA fragmentation	+	Danadevi et al., 2004
Nickel chloride	Human lung carcinoma A549 cells	Histone H4 acetylation	-	Broday et al., 2000
Nickel chloride	Human leukemia HL-60 cells	DNA fragmentation, cell death	+ +	Jia and Chen, 2008
Nickel arsenide	Mouse embryo C3H/10T1/2 Cl 8 cells	Cell transformation Chromosome aberrations	+ +	Clemens and Landolph, 2003

9.4.1 In vitro studies

Examination of the genotoxicity database for soluble nickel compounds indicated that they generally did not cause mutation in bacterial test systems. Positive results have been observed (1) in tests for single and double DNA strand breaks and/or crosslinks in both human and animal cells, (2) in tests for cell transformation, (3) in tests for sister chromatid exchanges and chromosomal aberrations in hamster and human cells, and (4) in tests for mutation at the HGPRT locus in animal cells (IARC, 1990).

Several studies reported that nickel compounds have the ability to enhance the cytotoxicity and mutagenicity of other DNA damaging agents such as ultra-violet light, benzo(a)pyrene, cis-platinum, and mitomycin C (Hartwig and Beyersmann, 1989; Christie, 1989; Rivedal and Sanner, 1980). Hartwig et al. (1994) showed that Ni^{2+} inhibited the removal of pyrimidine dimers and repair of DNA strand break in HeLa cells after exposure to ultra-violet light or X-rays. Hartmann and Hartwig (1998) demonstrated that the inhibition of DNA repair was effective at relatively low concentration, 50 μM Ni^{2+}, and partly reversible by the addition of Mg^{2+}. Based on these

observations, they suggested that Ni^{2+} disturbed DNA protein interactions essential for the DNA repair process by the displacement of essential metal ions.

Soluble nickel compounds can inhibit the normal DNA synthesis, impair or reduce the fidelity of DNA repair, and transform initiated cells in vitro. Basrur and Gilman (1967) and Swierenga and McLean (1985) showed that nickel chloride inhibited DNA synthesis in primary rat embryo cells and in rat liver epithelial cells. Costa et al. (1982) found that nickel chloride at 40-120 μM selectively blocked cell cycle progression in the S phase in Chinese hamster ovary cells.

Abbracchio et al. (1982) demonstrated that Chinese hamster ovary cells maintained in a minimal salts/glucose medium accumulated 10-fold more ^{63}Ni than did cells maintained in a minimal salts/glucose medium with 5 mM cysteine. The results were obtained after the removal of surface-associated radioactivity by treating the cells with trypsin. They also showed that supplementation of the salts/glucose medium with fetal bovine serum decreased in a concentration dependent fashion both the Ni^{2+} uptake and cytotoxicity.

Nieborer et al. (1984) demonstrated that chelation of Ni^{2+} by amino acids and proteins has a significant effect on the cellular uptake of Ni^{2+} in human B-lymphoblasts, human erythrocytes, and rabbit alveolar macrophages. They observed that addition of L-histidine or human serum albumin at physiological concentrations to the cell cultures reduced Ni^{2+} uptake by 70% -90%. The concentration of nickel used in the study was $7x10^{-8}$ M (or 4.1 μg/L); it was comparable to serum nickel levels observed in workers occupationally exposed to nickel.

Findings of Nieborer et al. (1984) and Abbracchio et al. (1982) indicate the important role of specific amino acids and proteins in regulating the uptake and cytotoxicity of Ni^{2+}. For this reason, when in vitro genotoxicity test results are compared, it is important to standardize the concentration of these chelating agents.

Zhuang et al. (1996) treated MRC-5 human lung fibroblast cells with crystalline Ni_3S_2 (0, 2.5, 5.0, 10.0, or 20 μg/cm^2) for four hours and DNA strand breaks measured by single cell electrophoresis (comet assay). All Ni-treated cells gave significantly increased tail lengths compared to the control ($P < 0.01$). A linear dose-response was observed up to 10 μg Ni/cm^2 (their Fig 2a). Significant leakage of lactate dehydrogenase was seen at 10 and 20 μg/cm^2 and increased activity of poly (ADP-ribose) polymerase (PADPRP) at 5.0 μg/cm^2 and above ($P < 0.01$). PADPRP is a nuclear enzyme associated with DNA damage and repair. PADPRP activity (pmol/μg DNA) was directly correlated with tail length (μm) in the comet assay ($R = 0.971$).

Lynn et al. (1997) studied the role of Ni^{2+} and ROS on enzymes of DNA repair in CHO cells in vitro. Nickel chloride exposure increased cellular oxidant levels in CHO cells in a dose-dependent manner between two and eight mM. When inhibitors of glutathione (BSO, buthione sulfoximine) or catalase (3ATA, 3-aminotriazole) were included with nickel chloride the cytotoxicity of Ni^{2+} was significantly enhanced. The effect was more pronounced in UV-irradiated cultures indicating that ROS were involved in the cytotoxic effect of nickel as well as the enhancing effect of nickel on UV cytotoxicity. The authors

tested the effect of H_2O_2 on Ni inhibition of DNA polymerase and ligation. In the presence of 0.1 mM $NiCl_2$ or 1.0 mM H_2O_2, the activities of DNA ligation were about 85% and 50% of control, respectively. The activity of DNA ligation decreased to 9.3% when cell extracts were treated with 0.1 mM $NiCl_2$ and then with 1.0 mM H_2O_2. This level was significantly lower than expected by simple additivity (χ^2 analysis).

This synergistic inhibition induced by Ni plus H_2O_2 was also observed in DNA polymerization in which activity fell to 46.5% after treatment with 0.1 mM $NiCl_2$ and 2.0 mM H_2O_2. The results indicate that DNA ligation is more sensitive to oxidant enhanced Ni inhibition than DNA polymerase. A 30-minute incubation with glutathione could completely remove the inhibition of Ni or recover ligation activity to 80% of control following H_2O_2 treatment or only 45% of control following Ni plus H_2O_2. Ni has a high binding affinity with cellular proteins ($K = 10^9\ M^{-1}$). The redox potential of Ni^{2+} is very high but can be lowered by binding to suitable ligands, such as the imidazole nitrogen of histidine. In the presence of oxidants such as H_2O_2 Ni^{2+}/Ni^{3+}, redox cycling can occur leading to the formation of free radicals such as •OH. Radical formation can lead to irreversible damage to proteins involved in DNA repair, replication, recombination and transcription and contribute to the toxic effects of nickel.

Mayer et al. (1998) tested Ni_3S_2 in a *lacI* transgenic BigBlue Rat 2 embryonic fibroblast cell line exposed for two hours to 0, 0.01, 0.04, or 0.17 mM Ni_3S_2. The mutation frequencies were 4(control), 7.2, 10.4, and 34.1 ($\times 10^{-5}$), respectively. However, molecular analysis in one-third of the mutants did not show DNA sequence change in the *lacI* gene despite loss of function. DNA damage as indicated by fragmentation in the comet assay was also seen in lung and nasal mucosa cells at 0.04 and 0.3 mM Ni_3S_2. Transgenic mice and rats were also exposed by inhalation for two hours (nose only) to 24-352 mg Ni_3S_2/m^3. Control animals were exposed to 8-126 mg $CaCO3/m^3$and sacrificed immediately after exposure. Transgenic test animals were sacrificed after an expression time of 14 days. Nasal mucosa and lung tissues were removed and frozen until analysis. The spontaneous mutation frequencies of the *lacZ* in mice or the *lacI* in rats was not significantly increased compared to controls in these tissues by exposure to 10 mg Ni_3S_2/kg bw and 6 mg Ni_3S_2/kg bw, respectively.

Iwitzki et al. (1998) studied the effect of nickel chloride on the induction and repair of O^6-methylguanine and N7-methylguanine after treatment with N-methyl-N-nitrosourea (MNU) in Chinese hamster ovary cells. The CHO cells were transfected with human O^6-methylguanine-DNA methyltransferase (MGMT) cDNA, and compared with MGMT-deficient parental cells. For N7-methylguanine repair, there was no marked difference in the kinetics of lesion removal with or without nickel. However, nickel (II) led to a significant decrease in repair of O^6-methylguanine lesions. Seventy-eight percent of O^6-methylguanine was repaired in 24 hours in the absence of nickel, while this was reduced to 48% with 250µ*M* Ni^{2+}. Nickel-induced inhibition of repair exhibited a dose-dependence in the 50-250 µM range. Repair inhibition was accompanied by an increase in MNU-induced cytotoxicity in nickel-treated cells but not in MGMT-deficient controls.

Kawanishi et al. (2001, 2002) described two separate mechanisms of oxidative DNA damage induced by Ni_3S_2 in studies with calf thymus DNA, cultured HeLa cells, or rats exposed intratracheally. With calf thymus DNA treated with Ni(II) and H_2O_2 they observed a time- and peroxide-dependent increase in 8-hydroxydeoxyguanosine (8-OH-dG). Ni(II) or H_2O_2 alone gave little or no increase in 8-OH-dG. With HeLa cells, incubation with Ni_3S_2 for 24 hr significantly increased 8-OH-dG in extracted cellular DNA. Similar incubations with Ni_2O_3 (black), NiO (green), or $NiSO_4$ did not induce 8-OH-dG formation. A significant increase in of 8-OH-dG was found in DNA extracted from lungs of 3 to 5 rats treated with 1.0 mg each of the nickel compounds intratracheally. The mean 8-OH-dG formation was Ni_3S_2 (2.57±0.87), Ni_2O_3 (black, 2.33±0.55), NiO (green, 2.33±0.61), $NiSO_4$ (1.65±0.97), and control (0.78±0.51) in units of 8-OH-dG/dG x 10^5. All mean increases were significantly greater than the control mean ($P < 0.05$). The results were interpreted by the authors as supporting a direct mode of DNA damage whereby Ni(II) enters the cells and then interacts with endogenous and/or Ni_3S_2-produced H_2O_2 to give reactive oxygen species that cause DNA damage. Additionally an indirect mode of oxidative DNA damage via inflammation is also supported. In this mode the sources of endogenous oxygen radicals are phagocytic cells such as neutrophils and macrophages. All of the nickel compounds can operate via the indirect mode while nickel subsulfide can also act directly.

Lei et al. (2001) measured DNA strand breaks, DNA-protein crosslinks, and telomerase activity in nickel-transformed BALB/c-3T3 cells in vitro. The transformed loci were induced by insoluble crystalline NiS (0.5 µg/cm^2), soluble $NiCl_2$ (50 µM) and $NiSO_4$ (100 µM). All three compounds showed statistically significant DNA strand breaks by the comet assay (single cell electrophoresis). The mean tail lengths of 100 comets were control 13.4, NiS 51.9, $NiCl_2$ 48.3, and $NiSO_4$ 42.2 µm, (all $P < 0.01$ vs. control). DNA-protein crosslinks were measured by ^{125}I-postlabelling techniques. Again all three nickel compounds gave significantly increased crosslinks compared to the control non-transformed cells 618, NiS 2414, $NiCl_2$ 1127, and $NiSO_4$ 988 cpm/µgDNA (all $P < 0.05$). In this case NiS was clearly much more active than the soluble nickel compounds. Telomerase activities were detected in all three nickel-transformed cells but the activity was much higher with NiS and $NiCl_2$ than with $NiSO_4$.

Ohshima (2003) studied genetic instability induced by nickel sulfate in V79 Chinese hamster cells. The cells were treated with 320 µM $NiSO_4$ for 24 hr at low cell density of 100 cells/100 mm diameter dish and clones selected from single surviving cells. When post-treatment cells were grown to 23-25 population doublings, the mutation frequency at the hypoxanthine phosphoribosyltransferase (HPRT) locus and chromosome aberration frequency of each clone were measured. Five out of 37 clones from Ni-treated cells showed increased frequencies of HPRT mutations ($\geq 1 \times 10^{-4}$), while only 1/37 control clones showed a mutation rate this high. Also, 17/37 clones from treated cells showed structural chromosomal aberrations vs. 3/37 for the controls. These included chromatid gaps and breaks, chromosome gaps and breaks, exchange, ring, and dicentric aberrations. The frequencies of chromosome gaps, ring, and dicentric aberrations were statistically significant compared to controls as was mean frequency of all aberrations ($P < 0.05$, *t*-test). Numerical aberrations were also observed in clones from Ni-treated cells: 8/37 for

aneuploidy and 11/37 for polyploidy. Only a few control clones showed such numerical aberrations. The authors conclude that nickel sulfate can induce genetic and chromosomal instability in V79 cells.

Oxidative DNA damage has been implicated as a contributing factor in neurodegeneration and heart disease as well as cancer and may figure in many degenerative diseases. Several studies to date have focused on the formation of the primary products of DNA oxidation: 7, 8-dihydro-8-oxoguanine (8-oxoG) and 8-hydroxy-2'-deoxyguanosine (8-OH-dG). Kelly et al. (2007) studied the oxidation of guanine, 8-oxoG and DNA by a Ni(II)/H_2O_2 system in vitro. They observed erratic oscillatory-like formation of 8-oxoG from free guanine and from DNA. Oxidation of 8-oxoG by Ni(II)/H_2O_2 led to guanidinohydantion (GH) or its oxidized analog (oxGH). The authors conclude that the instability of 8-oxoG (and presumably 8-OH-dG) in this system and its further oxidation products indicate a complex oxidative mechanism for guanine and unsuitability as a biomarker of DNA damage. However, it's not yet clear how quantitatively significant these "further" oxidative steps are under usual exposure scenarios.

Another problem with interpreting DNA adduct data is revealed by the study of Kaur and Dani (2003) on the relative nickel binding to RNA versus DNA. Female Sprague-Dawley rats (3 x 0.15 kg) were administered i.p. injections of $^{63}NiCl_2$. After 24 hr the animals were sacrificed and selected tissues removed for analysis. The subcellular distribution of ^{63}Ni in the liver, kidney, spleen and lungs was highest in the nucleus. About 10% to 50% of the nuclear radioactivity level was seen in the mitochondria, lysosomes, and microsomes. Further analysis of the nuclear fraction showed that in each tissue the large majority of ^{63}Ni label was associated with RNA rather than with DNA or nucleoproteins. The highest association observed was with kidney RNA. In vitro binding of $^{63}NiCl_2$ to DNA, denatured DNA, highly polymerized (HP) DNA, and RNA showed the maximum binding to RNA and HP DNA. Binding to DNA and denatured DNA was less than 25% of these values. Significant differences were observed between the infrared (IR) spectra of RNA and DNA incubated in vitro with $NiCl_2$, which also support the radiolabel findings. The authors postulate that Ni(II) may act by controlling gene expression post-transcriptionally via interaction with mRNA. Loss of mRNA has been reported in nickel-transformed cells (Salnikow et al., 1994).

Deng et al. (2006) observed that treatment of V79 cells with $NiCl_2$ after, but not before, exposure to benzo[a]pyrene (BaP) or its diol-epoxide (BPDE) metabolite led to significant enhancements of chromosome damage compared to control cells. Treatment of V79 cell for two hours with 0, 1, 5, 10, or 20 µg/mL of $NiCl_2$ resulted in proportions of aberrant cells of 0.75%, 0.75%, 1.0%, 1.3%, and 1.8 %, respectively. A similar value, 1.3% was obtained with 0.5 µg/mL BaP. Treatment of $NiCl_2$ at 5, 10, or 20 µg/mL after BaP exposure gave 9.3%, 12%, or 13% aberrant cells (all $P < 0.05$). The large majority of aberrations were chromosome breaks. The authors interpret the Ni-mediated potentiation of BaP genetic toxicity as a result of nickel inhibition of nucleotide excision repair (NER).

9.4.2 In vivo studies

The clastogenic potential of soluble nickel compounds has been shown in many in vivo studies. Sobti and Gill (1989) reported that oral administration of nickel sulfate (28 mg Ni/kg bw), nickel nitrate (23 mg Ni/kg bw), or nickel chloride (43 mg Ni/kg bw) to mice increased the frequency of micronuclei in the bone marrow at 6 and 30 hours after treatment. Details of the study were not reported and it was not clear how many animals were used in each experiment. Mohanty (1987) reported that intraperitoneal injections of nickel chloride at 6, 12, or 24 mg/kg bw increased the frequency of chromosomal aberrations in bone-marrow cells of Chinese hamsters. However, Mathur et al. (1978) observed that intraperitoneal injections of nickel sulfate at 3 and 6 mg/kg bw did not induce chromosomal aberrations in bone-marrow cells and spermatogonia of male albino rats. Saplakoglu et al. (1997) administered 44.4 mg nickel chloride/kg bw to rats via subcutaneous injections and did not observe increased levels of single-strand breaks in cultured lung, liver, or kidney cells.

Similarly, Deknudt and Leonard (1982) administered 25 mg/kg bw nickel chloride and 56 mg/kg nickel nitrate (about 50% of the LD_{50} in both cases) to mice by intraperitoneal injection and did not detect a significant increase of micronuclei in the bone marrow of the animals after 30 hours. Inhibition of DNA synthesis has been observed in vivo. Amlacher and Rudolph (1981) observed that intraperitoneal injections of nickel sulfate at 15 - 30% of the LD_{50} to CBA mice suppressed DNA synthesis in hepatic epithelial cells and in the kidney. Hui and Sunderman (1980) also reported that intramuscular injections of nickel chloride to rats at 20 mg Ni/kg bw inhibited DNA synthesis in the kidney.

Danadevi et al. (2004) administered $NiCl_2$ to 4-week old male Swiss mice. Eight groups of five animals each were given 0, 3.4, 6.8, 13.6, 27.2, 54.4, or 108.8 mg $NiCl_2$/kg bw by gavage. One group was given 25 mg cyclophosphamide/kg bw i.p. as a positive control. Blood was collected from each animal at 24, 48, and 72 hr, one week and two weeks post-treatment. DNA damage was assessed by single cell electrophoresis of leucocytes (comet assay). All doses produced significant dose-dependent DNA damage ($P < 0.05$) when compared to controls at 24, 48, 72 hr and one week. Clinical signs included loss in weight and feed intake at doses $\geq$ 13.6 mg $NiCl_2$/kg bw. From 72 hr post-treatment the mean comet lengths of all doses gradually decreased and after two weeks the lower doses ($\leq$13.6 mg/kg) were not significantly different from the negative controls.

Jia and Chen (2008) extended their study of antioxidant protection against nickel-induced DNA fragmentation to 40 male C57 mice and ascorbic acid (ASA) as antioxidant. Five groups of eight mice each were treated with a single daily i.p. injection for two weeks with 0, 2.0, 20.0 mg/kg-d $NiCl_2$, 2.0 + 5.0 mg/kg-d ASA, or 20.0 + 5.0 mg/kg-d ASA. DNA fragmentation and malondialdehyde (MDA) generation were measured in peripheral blood mononuclear cells (PBMC) and serum, respectively. Without ASA significant dose-dependent DNA fragmentation and MDA generation was observed. For DNA fragmentation the mean ($\pm$ SD, N = 8) for 0, 2, and 20 mg Ni/kg-d were 4.68 $\pm$ 0.89%, 9.83 $\pm$ 1.16%* and 15.25(1.91) %*, respectively (*$P < 0.01$). MDA in serum also showed a significant but shallower increase. Treatment of Ni + ASA showed slight, non-statistically significant, increases of MDA and DNA fragmentation. For the

latter the values were 4.68(0.89), 6.16(0.88), and 7.85(1.1), respectively. MDA values gave a shallower response. No trend tests were provided. The authors suggest the use of ascorbic acid to ameliorate the chronic toxic effects in individuals occupationally exposed to nickel compounds.

9.4.3 Mode of genotoxic action

A number of hypotheses have been proposed about the mechanisms that can explain the observed genotoxicity and transformation potential of soluble nickel compounds. Costa et al. (1982) and Sahu et al. (1995) showed that soluble nickel compounds affected cell growth by selectively blocking the S-phase of the cell cycle. Kasprzak (1991) and Sunderman (1989) suggested that most of the genotoxic characteristics of Ni^{2+} including DNA strand breaks, DNA-protein crosslinks, and chromosomal damage could be explained by the ability of Ni^{2+} to generate oxygen free radicals. While Ni^{2+} in the presence of inorganic ligands is resistant to oxidation, Ni^{2+} chelated with peptides has been shown to be able to catalyze reduction-oxidation reactions. Andrews et al. (1988) observed that certain peptides and proteins (especially those containing a histidine residue) form coordination complexes with Ni^{2+}. Many of these complexes have been shown to react with O_2 and/or H_2O_2 and generate oxygen free radicals (such as •OH) *in vitro* (Bossu et al., 1978; Inoue and Kawanishi, 1989; Torreilles and Guerin, 1990; Nieboer et al., 1984 and 1988). It is important to note that the major substrates for nickel mediated oxygen activation, O_2 and H_2O_2, are found in mammalian cells, including the nucleus (Peskin and Shlyahova, 1986).

Tkeshelashvili et al. (1993) showed that mutagenesis of Ni^{2+}in a bacterial test system could not only be enhanced by the addition of both hydrogen peroxide and a tripeptide glycyl-glycyl-L-histidine but also could be reduced by the addition of oxygen radical scavengers. Huang et al. (1993) treated Chinese hamster ovary cells with 0 to 5 mM nickel chloride and the precursor of fluorescence dye, 2, 7-dichlorofluorescin diacetate, and observed a significant increase of fluorescence in intact cells around the nuclear membranes. The effect was related to the concentration of the nickel chloride and was detectable at or below 1 mM. Since only strong oxidants, such as hydrogen peroxide and other organic hydroperoxides, can oxidize the nonfluorescent precursor to a fluorescent product, Huang et al. (1993) suggested that Ni^{2+} increased the level of such oxidants in intact cells.

Evidence of oxidative damage to cellular and genetic materials as a result of nickel administration has also been obtained from a number of *in vivo* studies. There are data indicating lipid peroxidation participates in the pathogenesis of acute nickel poisoning (Sunderman et al., 1985; Donskoy et al., 1986; Knight et al., 1986; Kasprzak et al., 1986 and Sunderman et al., 1987). Stinson et al. (1992) subcutaneously dosed rats with nickel chloride and observed increased DNA strand breaks and lipid peroxidation in the liver 4-13 hours after the treatment. Kasprzak et al. (1992) administered nickel acetate (5.3 mg Ni/kg bw) to pregnant rats by a single or two intraperitoneal injections and identified eleven oxidized purine and pyrimidine bases from the maternal and fetal liver and kidney tissues. Most of the products identified were typical hydroxyl radical-produced derivatives of DNA bases, suggesting a role for hydroxyl radical in the induction of their

formation by Ni^{2+}. In two other animal studies, Kasprzak et al. (1990 and 1992) also observed elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in the kidneys of rodents administered a single intraperitoneal injection of nickel acetate. Formation of 8-OH-dG is often recognized as one of the many characteristics of •OH attack on DNA.

Besides generating oxygen free radicals, Ni^{2+} can also weaken cellular defense against oxidative stresses. Donskoy et al. (1986) demonstrated that administration of soluble nickel compounds depleted free-radical scavengers (e.g., glutathione) or catalase, superoxide dismutase, glutathione peroxidase, or other enzymes that protect against free-radical injury in the treated animals.

Insoluble crystalline nickel compounds are generally found to be more potent in genetic toxicity assays than the soluble or amorphous forms of nickel. To find out the reason for this phenomenon, Harnett et al. (1982) compared the binding of ^{63}Ni to DNA, RNA, and protein isolated from cultured Chinese hamster ovary cells treated with either crystalline nickel sulfide (^{63}NiS) or a soluble nickel compound, $^{63}NiCl_2$ (both at 10 μg/mL). They reported that in the case of $^{63}NiCl_2$ treatment, cellular proteins contained about 100 times more bound ^{63}Ni than the respective RNA or DNA fractions; whereas in cells treated with crystalline ^{63}NiS, equivalent levels of nickel were associated with RNA, DNA, and protein. In absolute terms, RNA or DNA had 300 to 2,000 times more bound nickel following crystalline ^{63}NiS treatment compared to cells treated with $^{63}NiCl_2$. Fletcher et al. (1994) reported similar findings. Chinese hamster ovary cells were exposed to either water-soluble or slightly water-soluble salts. They observed relatively high nickel concentrations in the cytosol and very low concentrations in the nuclei of the cells exposed to the water-soluble salts. In contrast, they found relatively high concentrations of nickel in both the cytosol and the nuclei of the cells exposed to the slightly water-soluble salts.

Sen and Costa (1986) and Costa et al. (1994) theorized that this is because NiS and $NiCl_2$ are taken up by cells through different mechanisms. Ni^{2+} has a high affinity for protein relative to DNA; treatment of cells with soluble nickel compounds resulted in substantial binding of the metal ion to cytoplasmic proteins, with a small portion of the metal ion eventually reaching the nucleus. When cells are treated with crystalline nickel sulfide, the nickel containing particles were phagocytosed and delivered to sites near the nucleus. This mode of intracellular transport reduces the interaction of Ni^{2+} with cytoplasmic proteins and peptides.

To support their theory, Sen and Costa (1986) exposed Chinese hamster ovary cells to nickel chloride alone, nickel chloride-albumin complexes, nickel chloride-liposomes, and nickel chloride-albumin complexes encapsulated in liposomes. They found that at a given concentration (between 100 and 1,000 μM), cellular uptakes of nickel were 2-4 fold higher when the ovary cells were exposed to nickel chloride-liposomes or nickel chloride-albumin complexes encapsulated in liposomes than to nickel chloride alone or nickel chloride-albumin complexes. Even at comparable levels of cellular nickel (approximately 300 pmole $Ni/10^6$ cells), fragmentation of the heterochromatic long arm of the X chromosome was only observed in cells treated with nickel encapsulated in liposomes and not in those exposed to nickel or nickel-albumin. Based on these data,

they suggested that the higher genotoxic potency of crystalline nickel sulfide and nickel encapsulated in liposomes was not primarily due to the higher cellular nickel concentration, but rather to the way nickel ion was delivered into cells.

IARC (1980) suggested that cellular binding and uptake of nickel depend on the hydro- and lipophilic properties of the nickel complexes to which the cells are exposed. Nickel-complexing ligands, L-histidine, human serum albumin, D-penicillamine, and ethylenediaminetetraacetic acid, which form hydrophilic nickel complexes, inhibited the uptake of nickel by rabbit alveolar macrophages, human B-lymphoblasts, and human erythrocytes. Diethyldithiocarbamate and sodium pyridinethione, however, which form lipophilic nickel complexes, enhanced the cellular uptake of nickel. Several ideas and findings bearing on the mode of action of nickel genotoxicity have been integrated into a scheme proposed by NTP (1996a) and reproduced in Figure 2.

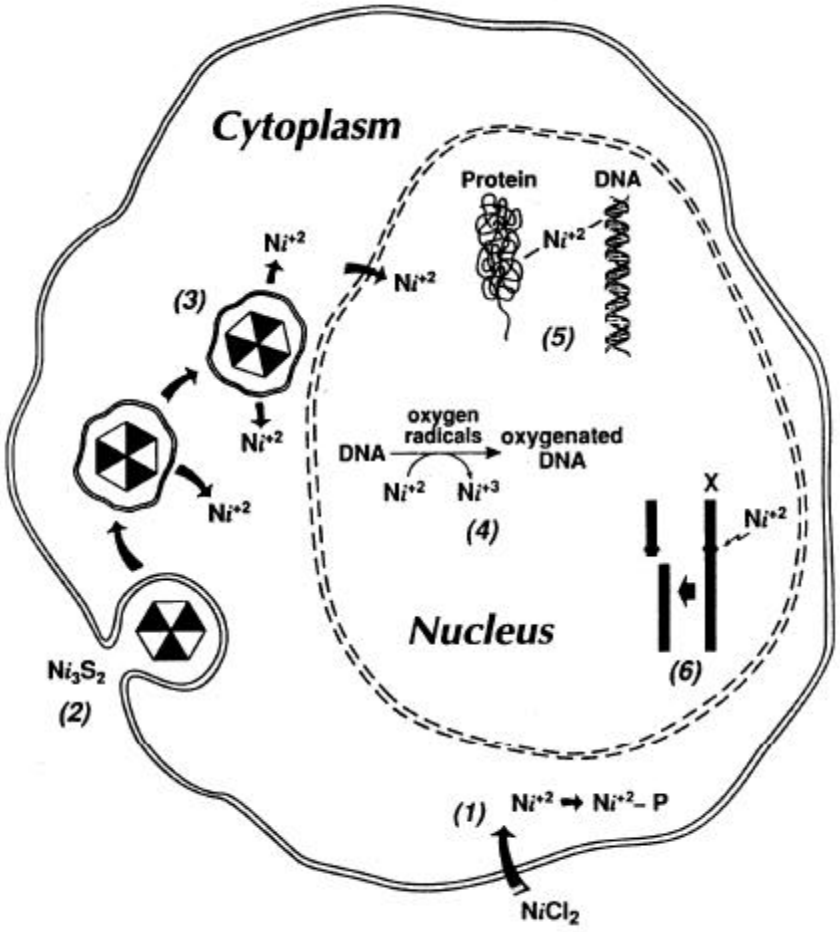

Figure 2. Possible mechanisms of nickel-induced genotoxicity (from NTP, 1996a).

1) Soluble nickel compounds such as nickel chloride diffuse into the cell; Ni^{2+} ions are rapidly bound to cytoplasmic proteins (P) (Lee et al., 1993). 2) Insoluble nickel compounds such as nickel subsulfide are phagocytized into the cell and move toward the nucleus (Costa et al., 1982).

3) Lysosomal breakdown of insoluble nickel compounds releases large quantities of Ni^{2+} ions that concentrate adjacent to the nuclear membrane (Costa and Heck, 1983). 4) Oxidative damage is induced in DNA by nickel ions bound to nuclear proteins ($Ni^{2+} \rightarrow Ni^{3+}$), releasing active oxygen species (Tkeshelashvili et al., 1993; Sugiyama, 1994). 5) DNA-protein crosslinks are produced by Ni^{2+} ions binding to heterochromatin (Lee et al., 1982; Patierno and Costa, 1985; Sen and Costa, 1986). 6) Binding of nickel ions to the heterochromatic regions of the long arm of the X chromosome, which may contain a senescence gene and a tumor suppressor gene, can cause deletion of all or part of this region, leading to an immortalization of the cell and clonal expansion (Conway and Costa, 1989; Klein et al., 1991).

In general nickel genotoxicity is the result of indirect mechanisms. Three mechanisms predominate: (1) interference with cellular redox regulation and induction of oxidative stress and possible oxidative DNA damage; (2) inhibition of major DNA repair systems resulting in genomic instability and accumulation of mutations; and (3) deregulation of cell proliferation by induction of signaling pathways or inactivation of growth controls including tumor suppressor genes (Beyersmann and Hartwig, 2008).

9.5 Chronic Toxicity Summary

The most important toxic effect seen in both nickel-exposed humans and experimental animals by inhalation is pneumotoxicity. In humans exposed occupationally this is expressed as nickel-induced asthma, pulmonary fibrosis, decreased lung function (FEV_1), and increased lung abnormalities revealed by radiography. In experimental animals adverse lung effects included inflammatory lesions, macrophage hyperplasia, alveolar proteinosis, and fibrosis (rats only), in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. Numerous other adverse effects at the cellular level were also seen contributing to cytotoxicity, genetic toxicity, immunotoxicity, and other metal-induced toxicity (Beyersmann and Hartwig, 2008; Rana, 2008). However, the most sensitive adverse effects were seen in the lung.

10. Derivation of Acute Reference Exposure Level (for a 1-hour exposure).

10.1 Acute Reference Exposure Level (aREL, for mild effects): 1.1 μg Ni/m³

Study	Cirla *et al*., 1985
Study population	7 volunteer metal plating workers with occupational asthma
Exposure method	inhalation of 0.3 mg/m³ $NiSO_4 \cdot 6H_2O$ (67 μg Ni/m³)
Critical effects	significant (> 15%) decrease in FEV_1
LOAEL	67 μg Ni/m³
NOAEL	not observed
Exposure duration	30 minutes
Extrapolated 1 hour concentration	33 μg Ni/m³ ((67 μg Ni/m³)* 0.5 hr = C * 1 h)
LOAEL uncertainty factor	10
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	(√10PD * 1PK)
Cumulative uncertainty factor	30
Reference Exposure Level	1.1 μg Ni/m³

The Cirla *et al*. (1985) study was selected as the basis for the acute or 1-hour REL since the study group included sensitive humans (asthmatics), thus reducing uncertainty for this effect. Although this study was conducted in asthmatic adults OEHHA expects that infants and children will be more sensitive to lung effects induced by airborne nickel. Thus we have applied an additional √10 uncertainty factor (UF) to account for additional pharmacodynamic differences between adults and infants and children with respect to lung or other adverse effects of nickel inhalation.

For comparison with the immunotoxicity of nickel, an extrapolation from the 2-hour LOAEL in mice of 250 μg Ni/m³ (Graham *et al*., 1978) to that of a 1-hour exposure was made using the time adjustment formula $C^n * T = K$, where n = 2. This yielded a 1-hour value of 354 μg/m³. Application of an uncertainty factor of 3000 to account for LOAEL, interspecies and individual variation would result in a 1-hour REL of 0.12 μg Ni/m³ and an 8-hour REL of 0.04 $\mu g/m^3$. This value should be reevaluated if human immunotoxicity data become available. The REL specifically does not apply to nickel carbonyl, which releases both nickel and carbon monoxide.

10.2 8-Hour Reference Exposure Level (8-hour REL):

Study	Graham *et al*., 1978 (supported by NTP. 1994c)
Study population	Swiss mice (female)
Exposure method	inhalation of 100 to 490 μg/m³ $NiCl_2$
Critical effects	depressed antibody response to sheep red blood cells
LOAEL	250 μg Ni/m³
BMDL	165 μg Ni/m^3 (-100 plaques/10^6 cells)
NOAEL	100 μg Ni/m^3 (this value is questionable)
Exposure duration	2 hours
Extrapolated 8 hour concentration	82 μg Ni/m³ ((165 μg Ni/m³)^2*2.0 hr = K, C^2*8 hr = K, C = 82.3 μg Ni/m^3)
BMDL uncertainty factor	√10
Interspecies uncertainty factor	10 (√10 PD * √10PK)
Intraspecies uncertainty factor	30 (10 PD * √10 PK)
Cumulative uncertainty factor	1000
Reference Exposure Level	0.08 μg Ni/m³

The studies and endpoints considered in deriving the 8-hour REL are summarized in Table 9. The 8-hour REL proposed is based on the Graham et al. (1978) study, the immunotoxicity endpoint and the 2 hr BMDL of 165 μg Ni/m^3. Where the 1-hour extrapolation yielded a value of 233 μg/m³ the 8-hour value was 82 μg/m^3. Using the cumulative uncertainty factor of 1000 yields an 8-hour REL of 0.08 μg/m^3. OEHHA anticipates that repeated exposures to airborne nickel will have a greater impact on infants and children than on adults due to its targeting of lung function and asthma inducing capability. This derivation is summarized below.

As an alternative or support to the Graham et al. (1978) study the NTP (1994c) bioassay results on non-neoplastic lung lesions could be considered. This study provides daily exposures of 6.2 hours for five days/week for durations of 16 days to 24 months (Table 10). The most consistent value presented was a NOAEL of 0.03 mg Ni/m^3 for alveolar macrophage hyperplasia in female rats (Table 10). This would give a daily value of 16.6 μg Ni/m^3 (30 μg Ni/m^3 x 6.2/8 hr/d x 5/7 days/wk) and with a cumulative uncertainty factor of 300 (UF_L = 1) the calculated 8-hour REL would be 0.055 μg Ni/m^3. The experimental exposures were 6.2 hours and repeated daily exposures were made. The advantage of the NTP study is multiple doses in two species and both sexes with extended durations of exposure. Daily exposures are close to eight hours and approximate the type of repeated exposures the 8-hour REL is intended to address. However, the Graham et al. (1978) study addresses a more sensitive toxic endpoint albeit with greater uncertainty due to study design limitations. The Ishihara et al. (2002) data on lung inflammation and mucus secretion endpoints generally fall in between the Graham et al. and the NTP studies in severity and duration of exposure, however the derived REL values appear to be consistent with the more severe lung and immunotoxicity effects evaluated.

Table 9. Studies and Toxic Endpoints Considered for the 8-Hour REL

Study	Endpoint	Criterion	Duration	8 hr Adjusted Value	Cumulative uncertainty factor	Proposed REL µg/m^3
Graham et al., 1978	Immunotoxicity	BMDL = 165 µg/m^3 (BMD_{100} = 284 µg/m^3)	2hr (single inhalation exposure)	82.3	1000	0.082
NTP 1994c	Lung toxicity	NOAEL = 0.03 mg/m^3	6.2 hr/d x 5 d/wk, 16 d – 7 mo	16.6 µg/m^3	300	0.055
Ishihara et al. 2002	Lung inflammation, total cells/µL in BALF	$BMDL_{1SD}$ = 5.5 µg (BMD_{1SD} = 9.8 µg)	5 hr/d x 5 d/wk x 1wk	19.6 µg/m^3	300	0.065
"	Lung inflammation, total protein in BALF, mg/mL	$BMDL_{1SD}$ = 18.6 µg (BMD_{1SD} = 26.9 µg)	5 hr/d x 5 d/wk x 1 wk	66.4 µg/m^3	300	0.22
"	Lung inflammation, total elastolytic activity in BALF	$BMDL_{1SD}$ = 50.0 µg (BMD_{1SD} = 53.0 µg)	5 hr/d x 5 d/wk x 1 wk	178 µg/m^3	300	0.60
"	Mucus secretion, sialic acid in BALF, µg/mL	$BMDL_{1SD}$ = 13.5 µg (BMD_{1SD} = 23.0 µg)	5 hr/d x 5 d/wk x 1wk	48.2 µg/m^3	300	0.16
Pandey & Srivastava, 2000	Decreased sperm motility percent	$BMDL_{1SD}$ = 2.91 mg $NiSO_4$/kg	1 oral dose/d x 5 d/wk x 5 wk	0.47 mg Ni/kg-d	1000	3.3
"	Increased Sperm Abnormalities percent	$BMDL_{1SD}$ = 0.46 mg $NiSO_4$/kg	1 oral dose/d x 5 d/wk x 5 wk	0.074 mg Ni/kg	1000	0.52
"	Increased Sperm Abnormalities percent	$BMDL_{1SD}$ = 0.34mg $NiCl_2$/kg	1 oral dose/d x 5 d/wk x 5 wk	0.060 mg Ni/kg	1000	0.42

Note: BALF = bronchoalveolar lavage fluid; for spermatotoxicity it was assumed that the hexahydrate salts were used, for the inhalation equivalent level it was assumed that only 50% of nickel would be absorbed via the inhalation route in addition to a 70 kg body weight and a 20 m^3/d inhalation rate (i.e. mouse µg/kg/d x 70 kg/20 m^3/d/0.5 = human µg/m^3.

Table 10. Non-neoplastic Lung Toxicity Observed with Inhalation of Nickel Sulfate (NTP, 1994c).

Effect	16 days (animals/dose group)*	13 weeks	7 months	15 months	24 months
Male Mice		**(N)OAEL or (L)OAEL mg Ni/m^3**			
Lung Inflammation	0.77L (5)	0.44N(10)	0.22N(5)	0.11N(5)	0.056N(61)
Alveolar Macrophage Hyperplasia		0.056N(10)	0.11N(5)	0.056N(5)	0.056N(61)
Fibrosis		0.22N(10)			
Female Mice					
Lung Inflammation	0.77L(5)	0.22N(10)	0.22N(5)	0.11N(5)	0.056L(60)
Alveolar Macrophage Hyperplasia		0.056N(10)	0.11N(5)	0.11N(5)	0.056L(60)
Fibrosis		0.22N(10)			
Male Rats					
Lung Inflammation	0.7L(5)	0.11N(10)	0.03L(5)	0.06N(5)	0.03N(53)
Alveolar Macrophage Hyperplasia		0.03L(10)	0.03N(5)	0.06N(5)	0.03N(53)
Fibrosis				0.11N(5)	0.03N(53
Female Rats					
Lung Inflammation	0.7L(5)	0.06N(10)	0.03N(5)	0.06N(5)	0.03N(53)
Alveolar Macrophage Hyperplasia		0.03L(10)	0.03N(5)	0.06N(5)	0.03N(53)
Fibrosis				0.11N(5)	0.03N(53)

*Note: animals exposed to $NiSO_4$ aerosol for 6.2 hr/day, 5days/week.

11. Derivation of Chronic Reference Exposure Levels (cRELs)

The studies conducted by NTP (1994a, b, & c) all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. For nickel sulfate the NOAEL and $BMDL_{05}$ for alveolar proteinosis are virtually identical for male or female rats. For extrapolation to humans the multi-pathway particle deposition model (MPPD) version two was used to derive a dosimetric adjustment factor (DAF) to calculate a human equivalent concentration (HEC, see Table 13). With a DAF of 0.27 the HEC was calculated as 1.5 µg/m^3. A cumulative uncertainty factor of 100 was used to derive a chronic REL of 0.015 µg/m^3. A supporting study is that of Berge and Skyberg

(2003) measuring pulmonary fibrosis in nickel refinery workers over a 22 year period. The authors found a weak but positive dose response for pulmonary fibrosis and cumulative nickel exposure expressed as (mg Ni/m^3)-yr. The best model fit to the data was obtained with the unadjusted data on soluble nickel of 0.35 (mg/m^3)-yr for the $BMDL_{01}$ (1% excess risk) (Table 11). Converting this value to a lifetime continuous value (8/24 hr x 5/7 days x 1/70 yr) gives 1.2 µg/m^3 equivalent and applying a 30-fold UF_H would give a supporting value for the cREL of 0.04 µg/m^3. The respiratory lesions observed in the Oller et al. (2008) chronic rat study with nickel metal powder give lower cREL values, particularly for alveolar proteinosis (0.004 µg Ni/m^3 female and 0.007 µg Ni/m^3 male), but the material is probably atypical of ambient air exposures.

Table 11. Benchmark Dose Analysis of Pulmonary Fibrosis in Nickel Refinery Workers (data from Berge & Skyberg, 2003)

Nickel type, cumulative dose	**Quantal response**	**Adjustment, goodness of fit χ^2, P**	**BMD_{01}**	**$BMDL_{01}$**
Soluble Ni: 0.03, 0.27, 1.03, and 4.32 (mg/m^3)-yr	6/254, 3/246, 13/283, 25/263	None, 2.21, 0.33	0.51	0.35
	6/254, 4/246, 12/283, 13/263	Age, smoking, asbestos, sulfidic Ni, 2.21, 0.33	1.38	0.69
	6/254, 4/246, 12/283, 16/263	Age, smoking, asbestos, 1.72, 0.42	0.98	0.56
Sulfidic Ni: 0.01, 0.08, 0.33, 1.73 (mg/m^3)-yr	4/264, 9/237, 15/282, 19/263	None, 3.91, 0.14	0.33	0.19
	4/264, 9/237, 11/282, (8/263)	Age, smoking, asbestos, soluble Ni, 3.27, 0.20; (1.87, 0.17)	No Value for full data set; (0.15 without top dose)	No Value for full data set; (0.063 without top dose)
	4/267, 10/237, 13/282, 12/263	Age, smoking, asbestos, 4.16, 0.125	0.95	0.34

Table 12 Benchmark Dose Analysis of Lung Effects Induced by NiO in Two-Year Studies (NTP, 1994a)*

Species, Sex, Endpoint, Quantal Response	Model	Goodness of Fit, X^2, p	BMD_{05}	$BMDL_{05}$
Rats, Male				
Bronchiolar hyperplasia 0/52,7/51,10/53,18/52	Quantal Linear	0.22, 0.89	0.15	0.004
Mice, Male				
Lung inflammation 0/57,21/67,34/66,55/69	Quantal Linear	0.09, 0.95	0.16	0.052
Alveolar proteinosis 0/57,12/67,22/66,43/69	Quantal Linear	0.09, 0.96	0.33	0.13
Mice, Female				
Lung inflammation 7/64,43/66,53/63,52/64	Multistage Cubic	0, 1.0	0.056	0.028
Alveolar proteinosis 0/64,8/66,17/63,29/64	Quantal Linear	0.14, 0.93	0.40	0.12

*Note: BMD and BMDL values are in mg Ni/m^3 continuous

Table 13. Lung Deposition of $NiSO_4 \cdot 6H_2O$ and NiO Particles Predicted by the Age-Specific MPPD Model (Version 2)*

Species/Age	$NiSO_4$(1) ADF	DAF	$NiSO_4$(2) ADF	DAF	NiO ADF	DAF
Rat, adult	0.1447	1.0	0.0718	1.0	0.1037	1.0
Human 3 months	0.3543	0.41	0.1847	0.18	0.4482	0.23
Human 3 years	0.2986	0.48	0.2276	0.23	0.3663	0.28
Human 9+ years	0.3931	0.37	0.3507	0.35	0.4546	0.23
Human 14 years	0.3536	0.41	0.215	0.22	0.14	0.26
Human 21 years	0.3070	0.47	0.3514	0.35	0.2656	0.39
Human mean		0.43		0.27		0.28

*Note: MPPD = Multi-Pathway Particle Deposition model run with particle concentration of 1 µg/m3, rat nasal breathing and human oronasal normal augmenter, ADF = airway deposition fraction (tracheobronchial plus alveolar), DAF = dosimetric adjustment factor (Human Equivalent Concentration = DAF x Animal Concentration);(1) $NiSO_4$ narrow particle distribution assumption, median mass aerodynamic diameter (MMAD) = 1.8 µm, geometric standard deviation(gsd) = 1.6 µm, density = 2.07 g/cm^3;(2) broad distribution assumption MMAD = 3.1 µm, gsd = 2.9 µm; NiO distribution MMAD = 3.29 µm, gsd = 2.04, density = 6.67 g/cm^3. The MPPD model was developed by the CIIT Center for Health Research, The National Institute of Public Health and the Environment, The Netherlands (RIVM), the Ministry of Housing Spatial Planning and the Environment, The Netherlands, and the National Institute for Occupational Safety and Health (NIOSH). See Brown et al. (2005) for model comparisons.

11.1. Nickel and Nickel Compounds (except nickel oxide)

Study	National Toxicology Program, 1994c
Study population	Male and female F344/N rats (52-53 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy
LOAEL	60 μg Ni/m^3 (as nickel sulfate hexahydrate)
NOAEL	30 μg Ni/m^3
$BMDL_{05}$	30.5 μg/m^3 (alveolar proteinosis, male and female mean)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	5.4 μg Ni/m^3 for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	1.5 μg Ni/m^3 for NOAEL group males (particulate with respiratory effects, DAF = 0.27 based on MMAD = 1.8 μm, gsd = 1.6 μm, density = 2.07 g/cm^3 by MPPD2 model)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	√10 (√10 PD * 1 PK)
Intraspecies uncertainty factor	30 (10 PD * √10 PK)
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.015 μg Ni/m^3

11.2 Nickel Oxide

For nickel oxide the benchmark dose analysis of the lung lesion data from NTP (1994a) gives an improved value of 117 μg Ni/m^3 for the $BMDL_{05}$. The results of the analysis are summarized in Table 10. The derivation of the chronic REL for NiO is similar to that for other nickel compounds shown above with only a slightly different DAF resulting in a proposed cREL for NiO of 0.06 μg/m^3 based on pulmonary inflammation in male and female mice.

Study	National Toxicology Program, 1994a
Study population	Male and female $B6C3F_1$ mice (57-69 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung: (1) active pulmonary inflammation, (2) alveolar proteinosis
LOAEL	500 mg Ni/m^3
NOAEL	Not observed
$BMDL_{05}$	117 μg Ni/m^3 (alveolar proteinosis)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	20.9 μg Ni/m^3 for LOAEL group (117 x 6/24 x 5/7)
Human equivalent concentration	5.8 μg Ni/m^3 for $BMDL_{05}$ for female mice (particulate with respiratory effects, DAF = 0.28 based on MMAD = 3.29 μm, gsd = 2.04 μm, density = 6.67 g/cm^3, by MPPD2 model)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	√10 (√10PD * 1PK)
Intraspecies uncertainty factor	30 (10 PD * √10 PK)
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.06 μg Ni/m^3

The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or animals; therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel subsulfide. The higher chronic REL value for nickel oxide of 0.06 μg/m^3 reflects these dose response differences. OEHHA therefore concludes that 0.06 μg/m^3 is an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of

nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

11.3 Data Strengths and Limitations for Development of the Chronic REL

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species. The toxicological response to various inhaled nickel compounds in children compared to adults is also an area of uncertainty addressed by a larger uncertainty factor for intra-individual variation (UF_H). Nickel targets the immune system and the lung, which are likely a more susceptible system and organ in exposed infants and children.

12. Oral Chronic Reference Level

In addition to being inhaled, airborne nickel can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic REL for nickel is also required.

Derivation of Oral Chronic Reference Exposure Level

Study	Smith *et al*., 1993
Study population	Rats (Long-Evans)
Exposure method	Drinking water
Critical effects	Perinatal mortality in two generation study
LOAEL	10 ppm (3.1 mg/kg-day)
NOAEL	Not observed in second breeding (G2), 50 ppm in first breeding (G1).
Exposure continuity	Continuous
Exposure duration	Lifetime
Average exposure	1.3 mg/kg-day
Human equivalent concentration	1.3 mg/kg-day
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Oral reference exposure level	0.0013 mg/kg-day

The oral REL for nickel used the same study used for OEHHA's Public Health Goal for drinking water. Smith et al. (1993) observed increased frequency of perinatal death at 10 ppm nickel chloride in the second breeding of a one generation study in rats. The oral REL derivation summarized above used uncertainty factors of 10 each for LOAEL to NOAEL, interspecies, and intraspecies extrapolations. The final value is 0.0013 mg Ni/kg-d or 1.3 µg Ni/kg-d. Haber et al. (2000) have proposed an oral reference dose of 8

µg Ni/kg-d based on albuminuria seen in female Wistar rats exposed to $NiSO_4$ for six months (Vsykocil et al., 1994). In our view the limitations of the Vsykocil et al. study, particularly the lack of a clear dose response, render it less acceptable than the Smith et al. study as the basis for a chronic oral REL.

13. Nickel as a Toxic Air Contaminant

There is a potential for high exposure to nickel and nickel compounds in view of its widespread occurrence and numerous uses. The adverse impacts on the respiratory and immune systems described in Section 5,6,8 and 9 (including asthma), and also the increased perinatal mortality and reduced birth weight observed in animal studies of reproductive toxicity (see Section 7.2), are among the types of effect leading to differential impacts on infants and children. OEHHA therefore recommends that nickel be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

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Appendix A

A.1 Berkeley Madonna Code for Sunderman et al. Human Oral Nickel Model.

```
METHOD RK4 {integration routine}

STARTTIME = 0
STOPTIME=24 {hours}
DT = 0.02 {step time or integration interval, i.e. 1200 steps total}

{Nickel biokinetic model of Sunderman et al. 1989; model units µg, hr}
{Nickel compartments, µg Ni initial values}
init Agi = 50*BW {Ni dose given in water 50 µg/kg body weight}
init Aserum = 0
init Aurine = 0
init Atissues = 0

{Model parameters, /hr unless otherwise specified}
Kf = 0.092 {zero-order rate constant for dietary absorption of nickel}
K01 = 0.28 {first-order rate constant for intestinal absorption of oral NiSO4 in water}
K10 = 0.21 {first-order rate constant for nickel excretion in urine}
K12 = 0.38 {first-order rate constant for nickel transfer from serum to tissues}
K21 = 0.08 {first-order rate constant for nickel transfer from tissues to serum}
BW = 70 {kg}

{Model differential equations calculate masses of nickel in respective compartments over
24 hours}
d/dt(Agi) = -Kf - K01*Agi
d/dt(Aserum) = Kf + K01*Agi - K10*Aserum - K12*Aserum + K21*Atissues
d/dt(Atissues) = K12*Aserum - K21*Atissues
d/dt(Aurine) =  K10*Aserum

Massbal = Agi + Aurine+ Aserum + Atissues {sum of model compartments equals dose
input}
```

A.2 Berkeley Madonna Code for Nickel keratinocyte Model of Franks et al.

```
METHOD RK4 {integration routine}

STARTTIME = 0
STOPTIME=24
DT = 0.02

{Model parameters}
dni = 2.62E-5 {rate of cell death due to nickel ions, /µM/hr}
bci = 0 {/hr, rate of cytokine release by nickel affected cells}
kn = 13.3 {/hr, rate of ion exchange}
un = 2.2 {unitless, partition coefficient}
dn = 0.00875 {/hr, rate of natural wastage of cells}
dc = 0.133 {/hr, rate of natural decay of cytokines within the media}
bcn = 6.25E-5 {µM/hr, rate of cytokine release by nickel affected cells}
n0 = 0.0165 {volume of cells, mL}
A0 = 100 {µM}
cpg = c*1.77E4*1000 {IL-1ά, pg/mL}
init c = 0 {initial concentration IL-1ά}
init Ai = 0 {initial intracellular Ni concentration}
init n = n0 {initial volume of keratinocytes}
init Ac = A0 {initial extracellular concentration of Ni}

{model differential equations}
d/dt(n) = -kd*n  {volume fraction of keratinocytes}
kd = dni*Ai + dn
d/dt(Ac) = -kn*n*(un*Ac - Ai) + kd*n*Ai {extracellular nickel}
d/dt(Ai) = kn*n*(un*Ac - Ai) - Kd*n*Ai {intracellular nickel}
d/dt(c) = bcn*n + bci*n*Ai - dc*(1-n)*c {IL-1ά cytokine release}
```

A.3 Intracellular Dosimetry Model of Inhaled Nickel Subsulfide.

METHOD RK4 {integration routine}

STARTTIME = 0
STOPTIME= 168 {hours}
DT = 0.01
DTOUT = 0.5

{Nickel mass µmoles Ni_3S_2}
init Agi = 0
init Asurf = Concn*Vmuc*0.23/MW
init Aionic = Concn*Vmuc*0.10/MW
init Aven = 0
init Avacuol = 0
init Acyto = 0
init Acytprot = 0
init Aperinuc = 0
init Aperinucytprot = 0
init Anucl = 0
init Anucprot = 0

{Concentrations, µmol/mL}
Csurf = Asurf/Vsurf
Cionic = Aionic/Vmuc
Ccyto = Acyto/Vcyto
Cperinuc = Aperinuc/Vperinuc
Cven = Aven/Vven
Cnucl = Anucl/Vnucl
Cnuni = 3*Cnucl

{Volumes, mL}
Vcyto = 0.54*Vtb
Vnucl = 0.06*Vtb
Vperinuc = 0.1*Vtb
Vtb =0.07*Vlu
Vlu = 0.014*BW
Vsurf = Vtb
Vionic = Vtb
Vven = 0.04*BW
Vmuc = 100 {mL}

{Model parameters}
VmiC = 10 {µmol/hr/μm^2}
Vmi = VmiC*2.4E11
Kmi = 1E9 {µmol/mL}

VmeC = 0.001 {µmol/hr/µm^2}
Vme = VmeC*2.4E11
Kme = 1E9{µmol/mL}
Kdm = 0.0001{/h}
Kdv = 0.0106 {/h}
PAcpC = 0.011{µm^2/hr}
PAcp = PAcpC
PApnC = 1.5
PApn = PApnC
ClpC = 1E-8 {mL/hr/cell}
Clp = ClpC*1E9 {/1E9 cells}
ClmC = 1.0E-11{mL/hr/cell}
Clm = ClmC*1E9 {/1E9 cells}
AbcC = 1E3{µmol/mL}
Abc = AbcC
AbpC = 1E3
Abp = AbpC
AbnC = 1E4
Abn = AbnC
Kbc = 1E9 {µmol/mL}
Kbn = 1E9
Kbp = 1E9
Frac = 0.08
Md = 0.1*MMAD
MMAD = 3.75 {µm}
Concn = 10 {µg/mL}
MW = 234.19
BW = 7E4
Protmg = 161{mg/1E9 cells}
Acytomg = Acyto/Protmg {µmol Ni/mg cytosol protein}

{Differential equations, µmol/hr}
d/dt(Agi) = Asurf*Clmc + Aionic*Clmc
d/dt(Asurf) = - Asurf*Kdm - Csurf*Clmc - Csurf*Clp
d/dt(Aionic) = Asurf*Kdm - Cionic*Vmi/(Kmi + Cionic) - Cionic*Clm
d/dt(Aven) = Ccyto*Vme/(Kme + Ccyto)
d/dt(Acyto) = Cionic*Vmi/(Kmi + Cionic) - Ccyto*Vme/(Kme + Ccyto) + Avacuol*Kdv*Frac - Ccyto*Abc/(Kbc + Ccyto) - Acyto*PAcp
d/dt(Avacuol) = Csurf*Clp - Avacuol*Kdv*Frac - Avacuol*Kdv*(1-Frac)
d/dt(Acytprot) = Ccyto*Abc/(Kbc + Ccyto)
d/dt(Aperinuc) = Avacuol*Kdv*(1-Frac) + Acyto*PAcp - Cperinuc*Abp/(Kbp + Cperinuc) - Aperinuc*PApn
d/dt(Aperinucytprot) = Cperinuc*Abp/(Kbc + Cperinuc)
d/dt(Anucl) = Aperinuc*PApn - Cnucl*Abn/(Kbn + Cnucl)
d/dt(Anucprot) = Cnucl*Abn/(Kbn + Cnucl)

A.4 PBPK Rat Model for NiO Inhalation Based on Teeguarden et al.

METHOD Stiff {integration routine}

STARTTIME = 0
STOPTIME= 8640 (12 months}
DT = 0.001
DTOUT = 0.25

{Draft PBPK model for nickel inhaled as nickel oxide; model loosely based on Teeguarden et al. 2007 Mn model w/ Pi's based on Ishimatsu et al.1995 and lung clearance based on Benson et al. 1994 and Tanaka et al. 1985}

{NiO in tissues, µg}
init Aart =0 {arterial blood}
init Aven = 0 {venous blood}
init Amusc = 0 {muscle shallow}
init Amuscdeep = 0 {muscle deep}
init Abone = 0
init Abonedeep =0
init Akid = 0 {kidney shallow}
init Akiddeep = 0 {kidney deep}
init Aliv = 0 {liver shallow}
init Alivdeep = 0 {liver deep}
init Alu = 0 {lung shallow}
init Alungdeep = 0 {lung deep}
init Alungdep = 0 {lung surface deposition}
init Anpdeep = 0 {nasopharynx deep}
init Anpdep = 0 {nasopharynx surface deposition}
init Anp = 0 {nasopharynx shallow}
init Agi = 0 {gastro-intestinal tract}
init Afeces = 0
init Aurine = 0

{Cardiac output, alveolar ventilation, body weight L/hr, kg}
BW = 0.325 {body weight}
Qtot = 14.6*BW^0.74 {cardiac output}
Qalv = 1.2*Qtot {alveolar ventilation}

{Blood flows, L/hr}
Qmusc = 0.534*Qtot
Qbone = 0.122*Qtot
Qkid = 0.141*Qtot
Qliv = 0.183*Qtot
Qnp = 0.01*Qtot

{Tissue volumes, L}
Vart = 0.0224*BW
Vblood = 0.0676*BW
Vmusc = 0.738*BW
Vbone = 0.021*BW
Vbonedeep = 0.052*BW
Vkid = 0.007*BW
Vliv = 0.034*BW
Vlu = 0.007*BW
Vnp = 0.0038*BW
Vtb = 0.01107*BW
Vpu = 0.01107*BW
Vven = 0.0452*BW
Vdeplu = Vtb + Vpu

{Concentrations µg Ni/L}
Cart = Cvlung {arterial concentration}
Cvmusc = Amusc/(Vmusc * Pmusc) {concentration leaving the muscle shallow compartment}
Cmusc = (Amuscdeep+Amusc)/Vmusc {total concentration in muscle}
Cvbone = Abone/(Vbone*Pbone)
Cbone = (Abonedeep + Abone)/Vbone
Cvkid = Akid/(Vkid*Pkid)
Ckid = (Akiddeep + Akid)/Vkid
Cvliv = Aliv/(Vliv*Pliv)
Cliv = (Alivdeep + Aliv)/Vliv
Cvnp = Anp/(Vnp*Pnp)
Cnp = (Anpdeep + Anp)/Vnp
Cvlung = Alu/(Vlu*Plung)
Clung = (Alungdeep + Alu)/Vlu
Cven = Aven/Vven {venous concentration}
Cvtot = (Qmusc*Cvmusc + Qkid*Cvkid + Qliv*Cvliv + Qbone*Cvbone + Qnp*Cvnp)/Qtot {mixed venous concentration}
Cair = IF TIME <= 140 THEN 600 ELSE 0 {140 hr exposure to 600 µg/m3}
Tvol = Qalv/0.6 {tidal volume}

{tissue/blood partition coefficients, unitless}
Pmusc = 0.8
Pbone = 1.0
Pkid = 16.0
Pliv = 2.0
Plung = 4.0
Pnp = 0.3

{Clearance rates, /hr}
Kf = 0.0001*BW^-0.25

Kinmusc = 0.017*BW^-0.25 {rate constants for nickel moving into and out of deep tissue compartments}
Kinbone = 0.105*BW^-0.25
Kinkid = 0.146*BW^-0.25
Kinliv = 0.621*BW^-0.25
Kinnp = 0.035*BW^-0.25
Kinlung = 0.035*BW^-0.25
Koutmusc = 0.0035*BW^-0.25
Koutbone = 0.085*BW^-0.25
Koutkid = 0.007*BW^-0.25
Koutliv = 0.015*BW^-0.25
Koutnp = 0.035*BW^-0.25
Koutlung = 0.0002*BW^-0.25
Kurine = 0.15 {kidney shallow to urine}
Kfeces = 0.5 {GI tract to feces}
Kai = 0.25 {GI tract to liver shallow}
Kbile = 0.05 {Liver to GI tract}
Kgi = 0.1 {respiratory tract to GI tract, i.e. swallowed particles mechanically removed from lung}
{rate constants for uptake from respiratory tract surface into shallow and deep compartments for lung and nasopharynx}
KdepSL = 2.0*BW^-0.25
KdepDL = 0.0*BW^-0.25
KdepSN = 0.2*BW^-0.25
KdepDN = 0.0*BW^-0.25

{fractional coeffs for deposited particles}
fdepNP = 0.2 {nasopharnyx}
fdepTB = 0.08 {tracheobroncheal}
fdepPu = 0.05 {pulmonary}
fdepLu = fdepTB + fdepPu

{differential equations}
d/dt(Abone) = Qbone*(Cart - Cvbone) - Kinbone*Cvbone*Vbone + Koutbone*Abonedeep
d/dt(Abonedeep) = Kinbone*Cvbone*Vbone - Koutbone*Abonedeep

d/dt(Amusc) = Qmusc*(Cart - Cvmusc) - Kinmusc*Cvmusc*Vmusc + Koutmusc*Amuscdeep
d/dt(Amuscdeep) = Kinmusc*Cvmusc*Vmusc - Koutmusc*Amuscdeep

d/dt(Akid) = Qkid*(Cart - Cvkid) - Kinkid*Cvkid*Vkid + Koutkid*Akiddeep
d/dt(Akiddeep) = Kinkid*Cvkid*Vkid - Koutkid*Akiddeep

d/dt(Alu) = Qtot*(Cvtot - Cvlung) - Kinlung*Cvlung*Vlu + Koutlung*Alungdeep + kdepSL*Alungdep

d/dt(Alungdeep) = Kinlung*Cvlung*Vlu - Koutlung*Alungdeep + kdepDL*Alungdep
d/dt(Alungdep) = fdepLu*Cair*Tvol - kdepDL*Alungdep - kdepSL*Alungdep - Alungdep*Kgi

d/dt(Aven) = Qmusc*Cvmusc + Qbone*Cvbone + Qkid*Cvkid + Qliv*Cvliv + Qnp*Cvnp - Qtot*Cven
d/dt(Aart) = Qtot*(Cvlung - Cart)

d/dt(Aliv) = Qliv*(Cart - Cvliv) - Kbile*Cvliv*Vliv - Kinliv*Cvliv*Vliv + Koutliv*Alivdeep - Aliv*Kbile

d/dt(Alivdeep) = Kinliv*Cvliv*Vliv - Koutliv*Alivdeep

d/dt(Anp) = Qnp*(Cart - Cvnp) - Kinnp*Cvnp*Vnp + Koutnp*Anpdeep + kdepSN*Anpdep
d/dt(Anpdeep) = kdepDN*Anpdep - Koutnp*Anpdeep + Kinnp*Cvnp*Vnp
d/dt(Anpdep) = fdepNP*Cair*Tvol - kdepDN*Anpdep - kdepSN*Anpdep - Anpdep*Kgi

d/dt(Agi) = Anpdep*Kgi + Alungdep*Kgi - Kai*Agi - Kfeces*Agi + Aliv*Kbile
d/dt(Afeces) = Kfeces*Agi
d/dt(Aurine) = Akid*Kurine

MASSBAL1 = Abone + Akid + Aliv + Anp + Amusc + Alu
MASSBAL2 = Abonedeep + Akiddeep + Alivdeep + Anpdeep + Amuscdeep + Alungdeep
MASSBAL3 = Anpdep + Alungdep
MASSBAL4 = Aurine + Afeces + Agi
MASSTOT = MASSBAL1 + MASSBAL2 + MASSBAL3 + MASSBAL4

Table A4. Comparison of Predicted and Observed Nickel Tissue Concentrations Twelve Months after a 140 Hours Exposure to NiO Aerosol.*

Tissue µg/L	8.0 mg/m^3 Model	Observed	O/P	0.6 mg/m^3 Model	Observed	O/P
Bone	5.95	ND		0.45	ND	
Kidneys	99.62	100 ± 90	1.00	7.47	80 ± 30	10.7
Liver	116.72	110 ± 70	0.94	8.75	50 ± 20	5.7
Nasopharynx	3.47	ND		0.26	ND	
Muscle	15.82	ND		1.19	ND	
Lung	285826	277000 ± 98000	0.97	21437	17000 ± 4000	0.79

***Note: NiO aerosol MADD = 1.2 µm, gsd = 2.2 µm. Model exposure was continuous for 140 hr, actual exposure was discontinuous over a one month period (not specified but probably about 6 hr/day x 5 days/week x 30days).**

A.5 Biokinetic Model of Uthus (1999) for Oral $NiCl_2$ in the Rat.

```
METHOD Stiff

STARTTIME = 0
STOPTIME= 10000 {minutes}
DT = 0.02
DTOUT = 10
{Uthus biokinetic model for 63Ni in the rat, Proc ND Acad Sci, 53:92-96(1999)}
{model compartments, ug Ni}
init GI_1 = 0.84 {ug at 12.7 uCi/ug Ni}
init GI_2 = 0
init GI_11 = 0
init Feces_3 = 0
init Blood_16 = 0
init Blood_15 = 0
init Blood_10 = 0
init Blood_4 = 0
init Liver_5 = 0
init Liver_6 = 0
init Liver_12 = 0
init Urine_9 = 0
init Urine_13 = 0
init Body_7 = 0
init Body_8 = 0
init Body_14 = 0

{mass transfer rate constants, /min}
K2_1 = 0.975
K3_11 = 0.000543
K4_1 = 0.025
K4_5 = 0.14
K4_7 = 0.3
K4_15 = 0.02
K5_4 = 0.155
K5_6 = 0.055
K6_5 = 0.05
K6_12 = 0.00003
K7_4 = 1.0
K7_8 = 0.005
K8_7 = 0.05
K8_14 = 0.0004
K9_13 = 0.0007
K10_4 = 0.0525
K11_2 = 0.001
K12_6 = 0.00175
K13_4 = 1.05
```

K14_8 = 0.0075
K15_10 = 0.066667
K15_16 = 0.0015
K16_15 = 0.01

{model differential equations, ug/min}
d/dt(GI_1) = -GI_1*K2_1 - GI_1*K4_1
d/dt(GI_2) = GI_1*K2_1 - GI_2*K11_2
d/dt(GI_11) = GI_2*K11_2 - GI_11*K3_11
d/dt(Feces_3) = GI_11*K3_11
d/dt(Blood_4) = GI_1*K4_1 - Blood_4*K5_4 + Liver_5*K4_5 - Blood_4*K10_4 + Blood_15*K4_15 - Blood_4*K13_4 - Blood_4*K7_4 + Body_7*K4_7
d/dt(Blood_10) = Blood_4*K10_4 - Blood_10*K15_10
d/dt(Blood_15) = Blood_10*K15_10 - Blood_15*K4_15 + Blood_16*K15_16
d/dt(Blood_16) = -Blood_16*K15_16 + Blood_15*K16_15
d/dt(Liver_5) = Blood_4*K5_4 - Liver_5*K4_5 -Liver_5*K6_5 + Liver_6*K5_6
d/dt(Liver_6) = Liver_5*K6_5 - Liver_6*K5_6 - Liver_6*K12_6 + Liver_12*K6_12
d/dt(Liver_12) = Liver_6*K12_6 - Liver_12*K6_12
d/dt(Urine_13) = Blood_4*K13_4 - Urine_13*K9_13
d/dt(Urine_9) = Urine_13*K9_13
d/dt(Body_7) = Blood_4*K7_4 - Body_7*K4_7 - Body_7*K8_7 + Body_8*K7_8
d/dt(Body_8) = Body_7*K8_7 - Body_8*K7_8 + Body_14*K8_14 - Body_8*K14_8
d/dt(Body_14) = Body_8*K14_8 - Body_14*K8_14

{Mass balance}
Mass_1 = GI_1 + GI_2 + GI_11 + Feces_3
Mass_2 = Blood_4 + Blood_10 + Blood_15 + Blood_16
Mass_3 = Liver_5 + Liver_6 + Liver_12
Mass_4 = Body_7 + Body_8 + Body_14
Mass_5 = Urine_13 + Urine_9
Mass_total = Mass_1 + Mass_2 + Mass_3 + Mass_4 + Mass_5
PCRECOV = Mass_total*100/0.84 {percent recovery of administered Ni}